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(54) Title: COMPOUNDS AND METHODS FOR IMMUNOTHERAPY AND IMMUNODIAGNOSIS OF PROSTATE CANCER			
(57) Abstract			
<p>Compounds and methods for treating and diagnosing prostate cancer are provided. The inventive compounds include polypeptides containing at least a portion of a prostate protein. Vaccines and pharmaceutical compositions for immunotherapy of prostate cancer comprising such polypeptides or DNA molecules encoding such polypeptides are also provided. The inventive polypeptides may also be used to generate antibodies useful for the diagnosis and monitoring of prostate cancer. Nucleic acid sequences for preparing probes, primers, and polypeptides are also provided.</p>			

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**COMPOUNDS AND METHODS FOR IMMUNOTHERAPY
AND IMMUNODIAGNOSIS OF PROSTATE CANCER**

TECHNICAL FIELD

5 The present invention relates generally to the treatment, diagnosis and monitoring of prostate cancer. The invention is more particularly related to polypeptides comprising at least a portion of a prostate protein. Such polypeptides may be used in vaccines and pharmaceutical compositions for treatment of prostate cancer. The polypeptides may also be used for the production of compounds, such as
10 antibodies, useful for diagnosing and monitoring the progression of prostate cancer, and possibly other tumor types, in a patient.

BACKGROUND OF THE INVENTION

15 Prostate cancer is the most common form of cancer among males, with an estimated incidence of 30% in men over the age of 50. Overwhelming clinical evidence shows that human prostate cancer has the propensity to metastasize to bone, and the disease appears to progress inevitably from androgen dependent to androgen refractory status, leading to increased patient mortality. This prevalent disease is currently the second leading cause of cancer death among men in the U.S.

20 In spite of considerable research into therapies for the disease, prostate cancer remains difficult to treat. Commonly, treatment is based on surgery and/or radiation therapy, but these methods are ineffective in a significant percentage of cases. Three prostate specific proteins - prostate specific antigen (PSA) and prostatic acid phosphatase (PAP) - have limited diagnostic and therapeutic potential. PSA levels do
25 not always correlate well with the presence of prostate cancer, being positive in a percentage of non-prostate cancer cases, including benign prostatic hyperplasia (BPH). Furthermore, PSA measurements correlate with prostate volume, and do not indicate the level of metastasis.

30 Accordingly, there remains a need in the art for improved vaccines and diagnostic methods for prostate cancer.

SUMMARY OF THE INVENTION

The present invention provides compounds and methods for immunotherapy and diagnosis of prostate cancer. In one aspect, polypeptides are 5 provided comprising at least an immunogenic portion of a prostate protein having a partial sequence as provided in SEQ ID Nos. 2 and 4-8, or a variant of such a protein that differs only in conservative substitutions and/or modifications.

In related aspects, DNA sequences encoding the above polypeptides, expression vectors comprising these DNA sequences and host cells transformed or 10 transfected with such expression vectors are also provided. In preferred embodiments, the host cells are selected from the group consisting of *E. coli*, yeast and mammalian cells.

The present invention also provides pharmaceutical compositions comprising one or more of the polypeptides of SEQ ID Nos. 1-8, 20, 21, 25-31 or 15 44-57, or nucleic acids of SEQ ID Nos. 9-19, 22-24 or 32-43, and a physiologically acceptable carrier. The invention also provides vaccines comprising one or more of such polypeptides or nucleic acids in combination with a non-specific immune response enhancer.

In yet another aspect, methods are provided for inhibiting the 20 development of prostate cancer in a patient, comprising administering an effective amount of one or more of the polypeptides of SEQ ID Nos. 1-8, 20, 21, 25-31 or 44-57, or nucleic acids of SEQ ID Nos. 9-19, 22-24 or 32-43 to a patient in need thereof.

In further aspects, methods are provided for detecting prostate cancer in a patient, comprising: (a) contacting a biological sample obtained from a patient with a 25 binding agent that is capable of binding to a polypeptide of SEQ ID Nos. 1-8, 20, 21, 25-31 or 44-57; and (b) detecting in the sample a protein or polypeptide that binds to the binding agent.

In related aspects, methods are provided for monitoring the progression of prostate cancer in a patient, comprising: (a) contacting a biological sample obtained 30 from a patient with a binding agent that is capable of binding to a polypeptide of SEQ

ID Nos. 1-8, 20, 21, 25-31 or 44-57; (b) determining in the sample an amount of a protein or polypeptide that binds to the binding agent; (c) repeating steps (a) and (b); and comparing the amounts of polypeptide detected in steps (b) and (c).

Within related aspects, the present invention provides antibodies, 5 preferably monoclonal antibodies, that bind to the polypeptides described above, as well as diagnostic kits comprising such antibodies, and methods of using such antibodies to inhibit the development of prostate cancer.

The present invention also provides methods for detecting prostate cancer comprising: (a) obtaining a biological sample from a patient; (b) contacting the 10 sample with at least two oligonucleotide primers in a polymerase chain reaction, at least one of the oligonucleotide primers being specific for a DNA sequence selected from the group consisting of SEQ ID Nos. 9-19, 22-24 and 32-43; and (c) detecting in the sample a DNA sequence that amplifies in the presence of the oligonucleotide primer. In one embodiment, the oligonucleotide primer comprises at least about 10 contiguous 15 nucleotides of a DNA sequence selected from the group consisting of SEQ ID Nos. 9-19, 22-24 and 32-43.

In a further aspect, the present invention provides a method for detecting prostate cancer in a patient comprising: (a) obtaining a biological sample from the patient; (b) contacting the sample with an oligonucleotide probe specific for a DNA 20 sequence selected from the group consisting of SEQ ID Nos. 9-19, 22-24 and 32-43; and (c) detecting in the sample a DNA sequence that hybridizes to the oligonucleotide probe. In one embodiment, the oligonucleotide probe comprises at least about 15 contiguous nucleotides of a DNA sequence selected from the group consisting of SEQ ID Nos. 9-19, 22-24 and 32-43.

25 These and other aspects of the present invention will become apparent upon reference to the following detailed description and attached drawings. All references disclosed herein are hereby incorporated by reference in their entirety as if each was incorporated individually.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 illustrates a Western blot analysis of sera obtained from rats immunized with rat prostate extract.

Fig. 2 illustrates a non-reduced SDS PAGE of the rat immunizing
5 preparation of Fig. 1.

Fig. 3 illustrates the binding of a putative human homologue of rat steroid binding protein to progesterone and to estramustine.

DETAILED DESCRIPTION OF THE INVENTION

10 As noted above, the present invention is generally directed to compositions and methods for the immunotherapy, diagnosis and monitoring of prostate cancer. The inventive compositions are generally polypeptides that comprise at least a portion of a human prostate protein, the protein demonstrating immunoreactivity with human prostate sera. Also included within the present invention are molecules (such as
15 an antibody or fragment thereof) that bind to the inventive polypeptides. Such molecules are referred to herein as "binding agents."

In particular, the subject invention discloses polypeptides comprising at least a portion of a human prostate protein provided in SEQ ID Nos. 2 and 4-8, or a variant of such a protein that differs only in conservative substitutions and/or
20 modifications. As used herein, the term "polypeptide" encompasses amino acid chains of any length, including full length proteins, wherein the amino acid residues are linked by covalent peptide bonds. Thus, a polypeptide comprising a portion of one of the above prostate proteins may consist entirely of the portion, or the portion may be present within a larger polypeptide that contains additional sequences. The additional
25 sequences may be derived from the native protein or may be heterologous, and such sequences may be immunoreactive and/or antigenic.

As used herein, an "immunogenic portion" of a human prostate protein is a portion that reacts either with sera derived from an individual inflicted with autoimmune prostatitis or with sera derived from a rat model of autoimmune prostatitis.
30 In other words, an immunogenic portion is capable of eliciting an immune response and

- as such binds to antibodies present within prostatitis sera. Autoimmune prostatitis may occur, for example, following treatment of bladder cancer by administration of *Bacillus Calmette-Guerin (BCG)*, an avirulent strain of *Mycobacterium bovis*. In the rat model of autoimmune prostatitis, rats are immunized with a detergent extract of rat prostate.
- 5 Sera from either of these sources may be used to react with the human prostate derived polypeptides described herein. Antibody binding assays may generally be performed using any of a variety of means known to those of ordinary skill in the art, as described, for example, in Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1988. For example, a polypeptide may be
- 10 immobilized on a solid support (as described below) and contacted with patient sera to allow binding of antibodies within the sera to the immobilized polypeptide. Unbound sera may then be removed and bound antibodies detected using, for example, ^{125}I -labeled Protein A.
- A "variant," as used herein, is a polypeptide that differs from the recited
- 15 polypeptide only in conservative substitutions and/or modifications, such that the immunotherapeutic, antigenic and/or diagnostic properties of the polypeptide or molecules that bind to the polypeptide, are retained. For prostate proteins with immunoreactive properties, variants may generally be identified by modifying one of the above polypeptide sequences, and evaluating the immunoreactivity of the modified
- 20 polypeptide. For prostate proteins useful for the generation of diagnostic binding agents, a variant may be identified by evaluating a modified polypeptide for the ability to generate antibodies that detect the presence or absence of prostate cancer. Such modified sequences may be prepared and tested using, for example, the representative procedures described herein.
- 25 As used herein, a "conservative substitution" is one in which an amino acid is substituted for another amino acid that has similar properties, such that one skilled in the art of peptide chemistry would expect the secondary structure and hydrophobic nature of the polypeptide to be substantially unchanged. In general, the following groups of amino acids represent conservative changes: (1) ala, pro, gly, glu,

asp, gln, asn, ser, thr; (2) cys, ser, tyr, thr; (3) val, ile, leu, met, ala, phe; (4) lys, arg, his; and (5) phe, tyr, trp, his.

Variants may also, or alternatively, contain other modifications, including the deletion or addition of amino acids that have minimal influence on the 5 antigenic properties, secondary structure and hydrophobic nature of the polypeptide. For example, a polypeptide may be conjugated to a signal (or leader) sequence at the N-terminal end of the protein which co-translationally or post-translationally directs transfer of the protein. The polypeptide may also be conjugated to a linker or other sequence for ease of synthesis, purification or identification of the polypeptide (e.g., 10 poly-His), or to enhance binding of the polypeptide to a solid support. For example, a polypeptide may be conjugated to an immunoglobulin Fc region.

Polypeptides having one of the sequences provided in SEQ ID Nos. 1 to 8, 20, 21 and 25-31 may be isolated from a suitable human prostate adenocarcinoma cell line, such as LnCap.fgc (ATCC No. 1740-CRL). LnCap.fgc is a prostate 15 adenocarcinoma cell line that is a particularly good representation of human prostate cancer. Like the human cancer, LnCap.fgc cells form progressively growing tumors as xenografts in SCID mice, respond to testosterone, secrete PSA and respond to the presence of bone marrow components (e.g., transferrin). In particular, the polypeptides may be isolated by expression screening of a LnCap.fgc cDNA library with human 20 prostatitis sera using techniques described, for example, in Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratories, Cold Spring Harbor, NY (and references cited therein), and as described in detail below. The polypeptides of SEQ ID No. 48 and 49 may be isolated from the LnCap/fgc cell line by screening with sera from the rat model of autoimmune prostatitis discussed above. The 25 polypeptides of SEQ ID Nos. 50-56 may be isolated from the LnCap/fgc cell line by screening with human prostatitis sera as described in detail in Example 4. The polypeptides of SEQ ID No. 44-47 may be isolated from human seminal fluid as described in detail in Example 2. Once a DNA sequence encoding a polypeptide is obtained, any of the above modifications may be readily introduced using standard 30 mutagenesis techniques, such as oligonucleotide-directed site-specific mutagenesis.

The polypeptides disclosed herein may also be generated by synthetic or recombinant means. Synthetic polypeptides having fewer than about 100 amino acids, and generally fewer than about 50 amino acids, may be generated using techniques well known to those of ordinary skill in the art. For example, such polypeptides may be 5 synthesized using any of the commercially available solid-phase techniques, such as the Merrifield solid-phase synthesis method, where amino acids are sequentially added to a growing amino acid chain. See Merrifield, *J. Am. Chem. Soc.* 85:2149-2146, 1963. Equipment for automated synthesis of polypeptides is commercially available from suppliers such as Applied BioSystems, Inc., (Foster City, CA), and may be operated 10 according to the manufacturer's instructions.

Alternatively, any of the above polypeptides may be produced recombinantly by inserting a DNA sequence that encodes the polypeptide into an expression vector and expressing the protein in an appropriate host. Any of a variety of expression vectors known to those of ordinary skill in the art may be employed to 15 express recombinant polypeptides of this invention. Expression may be achieved in any appropriate host cell that has been transformed or transfected with an expression vector containing a DNA molecule that encodes a recombinant polypeptide. Suitable host cells include prokaryotes, yeast and higher eukaryotic cells. Preferably, the host cells employed are *E. coli*, yeast or a mammalian cell line, such as CHO cells. The DNA 20 sequences expressed in this manner may encode naturally occurring polypeptides, portions of naturally occurring polypeptides, or other variants thereof.

In general, regardless of the method of preparation, the polypeptides disclosed herein are prepared in substantially pure form (*i.e.*, the polypeptides are homogenous as determined by amino acid composition and primary sequence analysis). 25 Preferably, the polypeptides are at least about 90% pure, more preferably at least about 95% pure and most preferably at least about 99% pure. In certain preferred embodiments, described in more detail below, the substantially pure polypeptides are incorporated into pharmaceutical compositions or vaccines for use in one or more of the methods disclosed herein.

Polypeptides of the present invention that comprise an immunogenic portion of a prostate protein may generally be used for immunotherapy of prostate cancer, wherein the polypeptide stimulates the patient's own immune response to prostate tumor cells. In further aspects, the present invention provides methods for

5 using one or more of the immunoreactive polypeptides of SEQ ID Nos. 1 to 8, 20, 21, 25-31 and 44-57 (or DNA encoding such polypeptides) for immunotherapy of prostate cancer in a patient. As used herein, a "patient" refers to any warm-blooded animal, preferably a human. A patient may be afflicted with a disease, or may be free of detectable disease. Accordingly, the above immunoreactive polypeptides may be used

10 to treat prostate cancer or to inhibit the development of prostate cancer. The polypeptides may be administered either prior to or following surgical removal of primary tumors and/or treatment by administration of radiotherapy and conventional chemotherapeutic drugs.

In these aspects, the polypeptide is generally present within a pharmaceutical composition and/or a vaccine. Pharmaceutical compositions may comprise one or more polypeptides, each of which may contain one or more of the above sequences (or variants thereof), and a physiologically acceptable carrier. The vaccines may comprise one or more of such polypeptides and a non-specific immune response enhancer, such as an adjuvant, biodegradable microsphere (e.g., polylactic galactide) or a liposome (into which the polypeptide is incorporated). Pharmaceutical compositions and vaccines may also contain other epitopes of prostate cell antigens, either incorporated into a combination polypeptide (*i.e.*, a single polypeptide that contains multiple epitopes) or present within a separate polypeptide.

Alternatively, a pharmaceutical composition or vaccine may contain

25 DNA encoding one or more of the above polypeptides, such that the polypeptide is generated *in situ*. In such pharmaceutical compositions and vaccines, the DNA may be present within any of a variety of delivery systems known to those of ordinary skill in the art, including nucleic acid expression systems, bacteria and viral expression systems. Appropriate nucleic acid expression systems contain the necessary DNA

30 sequences for expression in the patient (such as a suitable promoter). Bacterial delivery

systems involve the administration of a bacterium (such as *Bacillus-Calmette-Guerrin*) that expresses an epitope of a prostate cell antigen on its cell surface. In a preferred embodiment, the DNA may be introduced using a viral expression system (e.g., vaccinia or other pox virus, retrovirus, or adenovirus), which may involve the use of a
5 non-pathogenic (defective), replication competent virus. Suitable systems are disclosed, for example, in Fisher-Hoch et al., *PNAS* 86:317-321, 1989; Flexner et al., *Ann. N.Y. Acad. Sci.* 569:86-103, 1989; Flexner et al., *Vaccine* 8:17-21, 1990; U.S. Patent Nos. 4,603,112, 4,769,330, and 5,017,487; WO 89/01973; U.S. Patent No. 4,777,127; GB 2,200,651; EP 0,345,242; WO 91/02805; Berkner, *Biotechniques* 6:616-627, 1988;
10 Rosenfeld et al., *Science* 252:431-434, 1991; Kolls et al., *PNAS* 91:215-219, 1994; Kass-Eisler et al., *PNAS* 90:11498-11502, 1993; Guzman et al., *Circulation* 88:2838-2848, 1993; and Guzman et al., *Cir. Res.* 73:1202-1207, 1993. Techniques for incorporating DNA into such expression systems are well known to those of ordinary skill in the art. The DNA may also be "naked," as described, for example, in published
15 PCT application WO 90/11092, and Ulmer et al., *Science* 259:1745-1749, 1993, reviewed by Cohen, *Science* 259:1691-1692, 1993. The uptake of naked DNA may be increased by coating the DNA onto biodegradable beads, which are efficiently transported into the cells.

Routes and frequency of administration, as well as dosage, will vary
20 from individual to individual and may parallel those currently being used in immunotherapy of other diseases. In general, the pharmaceutical compositions and vaccines may be administered by injection (e.g., intracutaneous, intramuscular, intravenous or subcutaneous), intranasally (e.g., by aspiration) or orally. Between 1 and 10 doses may be administered over a 3-24 week period. Preferably, 4 doses are
25 administered, at an interval of 3 months, and booster administrations may be given periodically thereafter. Alternate protocols may be appropriate for individual patients. A suitable dose is an amount of polypeptide or DNA that is effective to raise an immune response (cellular and/or humoral) against prostate tumor cells in a treated patient. A suitable immune response is at least 10-50% above the basal (i.e., untreated) level. In
30 general, the amount of polypeptide present in a dose (or produced *in situ* by the DNA in

a dose) ranges from about 1 pg to about 100 mg per kg of host, typically from about 10 pg to about 1 mg, and preferably from about 100 pg to about 1 µg. Suitable dose sizes will vary with the size of the patient, but will typically range from about 0.01 mL to about 5 mL.

5 While any suitable carrier known to those of ordinary skill in the art may be employed in the pharmaceutical compositions of this invention, the type of carrier will vary depending on the mode of administration. For parenteral administration, such as subcutaneous injection, the carrier preferably comprises water, saline, alcohol, a fat, a wax and/or a buffer. For oral administration, any of the above carriers or a solid carrier, 10 such as mannitol, lactose, starch, magnesium stearate, sodium saccharine, talcum, cellulose, glucose, sucrose, and/or magnesium carbonate, may be employed. Biodegradable microspheres (e.g., polylactic glycolide) may also be employed as carriers for the pharmaceutical compositions of this invention. Suitable biodegradable microspheres are disclosed, for example, in U.S. Patent Nos. 4,897,268 and 5,075,109.

15 Any of a variety of non-specific immune response enhancers may be employed in the vaccines of this invention. For example, an adjuvant may be included. Most adjuvants contain a substance designed to protect the antigen from rapid catabolism, such as aluminum hydroxide or mineral oil, and a nonspecific stimulator of immune response, such as lipid A, *Bordetella pertussis* or *Mycobacterium tuberculosis*. 20 Such adjuvants are commercially available as, for example, Freund's Incomplete Adjuvant and Complete Adjuvant (Disco Laboratories, Detroit, MI) and Merck Adjuvant 65 (Merck and Company, Inc., Rahway, NJ).

25 Polypeptides disclosed herein may also be employed in *ex vivo* treatment of prostate cancer. For example, cells of the immune system, such as T cells, may be isolated from the peripheral blood of a patient, using a commercially available cell separation system, such as CellPro Incorporated's (Bothell, WA) CEPRATETM system (see U.S. Patent No. 5,240,856; U.S. Patent No. 5,215,926; WO 89/06280; WO 91/16116 and WO 92/07243). The separated cells are stimulated with one or more of the immunoreactive polypeptides contained within a delivery vehicle, such as a 30 microsphere, to provide antigen-specific T cells. The population of tumor antigen-

specific T cells is then expanded using standard techniques and the cells are administered back to the patient.

Polypeptides of the present invention may also, or alternatively, be used to generate binding agents, such as antibodies or fragments thereof, that are capable of
5 detecting metastatic human prostate tumors.

Binding agents of the present invention may generally be prepared using methods known to those of ordinary skill in the art, including the representative procedures described herein. Binding agents are capable of differentiating between patients with and without prostate cancer, using the representative assays described
10 herein. In other words, antibodies or other binding agents raised against a prostate protein, or a suitable portion thereof, will generate a signal indicating the presence of primary or metastatic prostate cancer in at least about 20% of patients afflicted with the disease, and will generate a signal indicating the absence of the disease in at least about 90% of individuals without primary or metastatic prostate cancer. Suitable portions of
15 such prostate proteins are portions that are able to generate a binding agent that indicates the presence of primary or metastatic prostate cancer in substantially all (*i.e.*, at least about 80%, and preferably at least about 90%) of the patients for which prostate cancer would be indicated using the full length protein, and that indicate the absence of prostate cancer in substantially all of those samples that would be negative when tested
20 with full length protein. The representative assays described below, such as the two-antibody sandwich assay, may generally be employed for evaluating the ability of a binding agent to detect metastatic human prostate tumors.

The ability of a polypeptide prepared as described herein to generate antibodies capable of detecting primary or metastatic human prostate tumors may
25 generally be evaluated by raising one or more antibodies against the polypeptide (using, for example, a representative method described herein) and determining the ability of such antibodies to detect such tumors in patients. This determination may be made by assaying biological samples from patients with and without primary or metastatic prostate cancer for the presence of a polypeptide that binds to the generated antibodies.
30 Such test assays may be performed, for example, using a representative procedure

described below. Polypeptides that generate antibodies capable of detecting at least 20% of primary or metastatic prostate tumors by such procedures are considered to be able to generate antibodies capable of detecting primary or metastatic human prostate tumors. Polypeptide specific antibodies may be used alone or in combination to
5 improve sensitivity.

Polypeptides capable of detecting primary or metastatic human prostate tumors may be used as markers for diagnosing prostate cancer or for monitoring disease progression in patients. In one embodiment, prostate cancer in a patient may be diagnosed by evaluating a biological sample obtained from the patient for the level of
10 one or more of the above polypeptides, relative to a predetermined cut-off value. As used herein, suitable "biological samples" include blood, sera, urine and/or prostate secretions.

The level of one or more of the above polypeptides may be evaluated using any binding agent specific for the polypeptide(s). A "binding agent," in the
15 context of this invention, is any agent (such as a compound or a cell) that binds to a polypeptide as described above. As used herein, "binding" refers to a noncovalent association between two separate molecules (each of which may be free (*i.e.*, in solution) or present on the surface of a cell or a solid support), such that a "complex" is formed. Such a complex may be free or immobilized (either covalently or
20 noncovalently) on a support material. The ability to bind may generally be evaluated by determining a binding constant for the formation of the complex. The binding constant is the value obtained when the concentration of the complex is divided by the product of the component concentrations. In general, two compounds are said to "bind" in the context of the present invention when the binding constant for complex formation
25 exceeds about 10³ L/mol. The binding constant may be determined using methods well known to those of ordinary skill in the art.

Any agent that satisfies the above requirements may be a binding agent. For example, a binding agent may be a ribosome with or without a peptide component, an RNA molecule or a peptide. In a preferred embodiment, the binding partner is an
30 antibody, or a fragment thereof. Such antibodies may be polyclonal, or monoclonal. In

addition, the antibodies may be single chain, chimeric, CDR-grafted or humanized. Antibodies may be prepared by the methods described herein and by other methods well known to those of skill in the art.

There are a variety of assay formats known to those of ordinary skill in the art for using a binding partner to detect polypeptide markers in a sample. See, e.g., Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, 1988. In a preferred embodiment, the assay involves the use of binding partner immobilized on a solid support to bind to and remove the polypeptide from the remainder of the sample. The bound polypeptide may then be detected using a second binding partner that contains a reporter group. Suitable second binding partners include antibodies that bind to the binding partner/polypeptide complex. Alternatively, a competitive assay may be utilized, in which a polypeptide is labeled with a reporter group and allowed to bind to the immobilized binding partner after incubation of the binding partner with the sample. The extent to which components of the sample inhibit the binding of the labeled polypeptide to the binding partner is indicative of the reactivity of the sample with the immobilized binding partner.

The solid support may be any material known to those of ordinary skill in the art to which the antigen may be attached. For example, the solid support may be a test well in a microtiter plate or a nitrocellulose or other suitable membrane. Alternatively, the support may be a bead or disc, such as glass, fiberglass, latex or a plastic material such as polystyrene or polyvinylchloride. The support may also be a magnetic particle or a fiber optic sensor, such as those disclosed, for example, in U.S. Patent No. 5,359,681. The binding agent may be immobilized on the solid support using a variety of techniques known to those of skill in the art, which are amply described in the patent and scientific literature. In the context of the present invention, the term "immobilization" refers to both noncovalent association, such as adsorption, and covalent attachment (which may be a direct linkage between the antigen and functional groups on the support or may be a linkage by way of a cross-linking agent). Immobilization by adsorption to a well in a microtiter plate or to a membrane is preferred. In such cases, adsorption may be achieved by contacting the binding agent,

in a suitable buffer, with the solid support for a suitable amount of time. The contact time varies with temperature, but is typically between about 1 hour and about 1 day. In general, contacting a well of a plastic microtiter plate (such as polystyrene or polyvinylchloride) with an amount of binding agent ranging from about 10 ng to about 5 10 µg, and preferably about 100 ng to about 1 µg, is sufficient to immobilize an adequate amount of binding agent.

Covalent attachment of binding agent to a solid support may generally be achieved by first reacting the support with a bifunctional reagent that will react with both the support and a functional group, such as a hydroxyl or amino group, on the 10 binding agent. For example, the binding agent may be covalently attached to supports having an appropriate polymer coating using benzoquinone or by condensation of an aldehyde group on the support with an amine and an active hydrogen on the binding partner (see, e.g., Pierce Immunotechnology Catalog and Handbook, 1991, at A12-A13).

15 In certain embodiments, the assay is a two-antibody sandwich assay. This assay may be performed by first contacting an antibody that has been immobilized on a solid support, commonly the well of a microtiter plate, with the sample, such that polypeptides within the sample are allowed to bind to the immobilized antibody. Unbound sample is then removed from the immobilized polypeptide-antibody 20 complexes and a second antibody (containing a reporter group) capable of binding to a different site on the polypeptide is added. The amount of second antibody that remains bound to the solid support is then determined using a method appropriate for the specific reporter group.

More specifically, once the antibody is immobilized on the support as 25 described above, the remaining protein binding sites on the support are typically blocked. Any suitable blocking agent known to those of ordinary skill in the art, such as bovine serum albumin or Tween 20™ (Sigma Chemical Co., St. Louis, MO). The immobilized antibody is then incubated with the sample, and polypeptide is allowed to bind to the antibody. The sample may be diluted with a suitable diluent, such as 30 phosphate-buffered saline (PBS) prior to incubation. In general, an appropriate contact

- time (*i.e.*, incubation time) is that period of time that is sufficient to detect the presence of polypeptide within a sample obtained from an individual with prostate cancer. Preferably, the contact time is sufficient to achieve a level of binding that is at least about 95% of that achieved at equilibrium between bound and unbound polypeptide.
- 5 Those of ordinary skill in the art will recognize that the time necessary to achieve equilibrium may be readily determined by assaying the level of binding that occurs over a period of time. At room temperature, an incubation time of about 30 minutes is generally sufficient.

Unbound sample may then be removed by washing the solid support

10 with an appropriate buffer, such as PBS containing 0.1% Tween 20TM. The second antibody, which contains a reporter group, may then be added to the solid support. Preferred reporter groups include enzymes (such as horseradish peroxidase), substrates, cofactors, inhibitors, dyes, radionuclides, luminescent groups, fluorescent groups and biotin. The conjugation of antibody to reporter group may be achieved using standard

15 methods known to those of ordinary skill in the art.

The second antibody is then incubated with the immobilized antibody-polypeptide complex for an amount of time sufficient to detect the bound polypeptide. An appropriate amount of time may generally be determined by assaying the level of binding that occurs over a period of time. Unbound second antibody is then removed

20 and bound second antibody is detected using the reporter group. The method employed for detecting the reporter group depends upon the nature of the reporter group. For radioactive groups, scintillation counting or autoradiographic methods are generally appropriate. Spectroscopic methods may be used to detect dyes, luminescent groups and fluorescent groups. Biotin may be detected using avidin, coupled to a different

25 reporter group (commonly a radioactive or fluorescent group or an enzyme). Enzyme reporter groups may generally be detected by the addition of substrate (generally for a specific period of time), followed by spectroscopic or other analysis of the reaction products.

To determine the presence or absence of prostate cancer, the signal

30 detected from the reporter group that remains bound to the solid support is generally

compared to a signal that corresponds to a predetermined cut-off value. In one preferred embodiment, the cut-off value is the average mean signal obtained when the immobilized antibody is incubated with samples from patients without prostate cancer. In general, a sample generating a signal that is three standard deviations above the 5 predetermined cut-off value is considered positive for prostate cancer. In an alternate preferred embodiment, the cut-off value is determined using a Receiver Operator Curve, according to the method of Sackett et al., *Clinical Epidemiology: A Basic Science for Clinical Medicine*, Little Brown and Co., 1985, p. 106-7. Briefly, in this embodiment, the cut-off value may be determined from a plot of pairs of true positive rates (*i.e.*, 10 sensitivity) and false positive rates (100%-specificity) that correspond to each possible cut-off value for the diagnostic test result. The cut-off value on the plot that is the closest to the upper left-hand corner (*i.e.*, the value that encloses the largest area) is the most accurate cut-off value, and a sample generating a signal that is higher than the cut-off value determined by this method may be considered positive. Alternatively, the cut- 15 off value may be shifted to the left along the plot, to minimize the false positive rate, or to the right, to minimize the false negative rate. In general, a sample generating a signal that is higher than the cut-off value determined by this method is considered positive for prostate cancer.

In a related embodiment, the assay is performed in a flow-through or 20 strip test format, wherein the antibody is immobilized on a membrane, such as nitrocellulose. In the flow-through test, polypeptides within the sample bind to the immobilized antibody as the sample passes through the membrane. A second, labeled antibody then binds to the antibody-polypeptide complex as a solution containing the second antibody flows through the membrane. The detection of bound second antibody 25 may then be performed as described above. In the strip test format, one end of the membrane to which antibody is bound is immersed in a solution containing the sample. The sample migrates along the membrane through a region containing second antibody and to the area of immobilized antibody. Concentration of second antibody at the area of immobilized antibody indicates the presence of prostate cancer. Typically, the 30 concentration of second antibody at that site generates a pattern, such as a line, that can

be read visually. The absence of such a pattern indicates a negative result. In general, the amount of antibody immobilized on the membrane is selected to generate a visually discernible pattern when the biological sample contains a level of polypeptide that would be sufficient to generate a positive signal in the two-antibody sandwich assay, in 5 the format discussed above. Preferably, the amount of antibody immobilized on the membrane ranges from about 25 ng to about 1 μ g, and more preferably from about 50 ng to about 500 ng. Such tests can typically be performed with a very small amount of biological sample.

Of course, numerous other assay protocols exist that are suitable for use 10 with the antigens or antibodies of the present invention. The above descriptions are intended to be exemplary only.

In another embodiment, the above polypeptides may be used as markers for the progression of prostate cancer. In this embodiment, assays as described above for the diagnosis of prostate cancer may be performed over time, and the change in the 15 level of reactive polypeptide(s) evaluated. For example, the assays may be performed every 24-72 hours for a period of 6 months to 1 year, and thereafter performed as needed. In general, prostate cancer is progressing in those patients in whom the level of polypeptide detected by the binding agent increases over time. In contrast, prostate cancer is not progressing when the level of reactive polypeptide either remains constant 20 or decreases with time.

Antibodies for use in the above methods may be prepared by any of a variety of techniques known to those of ordinary skill in the art. See, e.g., Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, 1988. In one such technique, an immunogen comprising the antigenic polypeptide is initially injected 25 into any of a wide variety of mammals (e.g., mice, rats, rabbits, sheep and goats). In this step, the polypeptides of this invention may serve as the immunogen without modification. Alternatively, particularly for relatively short polypeptides, a superior immune response may be elicited if the polypeptide is joined to a carrier protein, such as bovine serum albumin or keyhole limpet hemocyanin. The immunogen is injected 30 into the animal host, preferably according to a predetermined schedule incorporating

one or more booster immunizations, and the animals are bled periodically. Polyclonal antibodies specific for the polypeptide may then be purified from such antisera by, for example, affinity chromatography using the polypeptide coupled to a suitable solid support.

5 Monoclonal antibodies specific for the antigenic polypeptide of interest may be prepared, for example, using the technique of Kohler and Milstein, *Eur. J. Immunol.* 6:511-519, 1976, and improvements thereto. Briefly, these methods involve the preparation of immortal cell lines capable of producing antibodies having the desired specificity (*i.e.*, reactivity with the polypeptide of interest). Such cell lines may
10 be produced, for example, from spleen cells obtained from an animal immunized as described above. The spleen cells are then immortalized by, for example, fusion with a myeloma cell fusion partner, preferably one that is syngeneic with the immunized animal. A variety of fusion techniques may be employed. For example, the spleen cells and myeloma cells may be combined with a nonionic detergent for a few minutes and
15 then plated at low density on a selective medium that supports the growth of hybrid cells, but not myeloma cells. A preferred selection technique uses HAT (hypoxanthine, aminopterin, thymidine) selection. After a sufficient time, usually about 1 to 2 weeks, colonies of hybrids are observed. Single colonies are selected and tested for binding activity against the polypeptide. Hybridomas having high reactivity and specificity are
20 preferred.

Monoclonal antibodies may be isolated from the supernatants of growing hybridoma colonies. In addition, various techniques may be employed to enhance the yield, such as injection of the hybridoma cell line into the peritoneal cavity of a suitable vertebrate host, such as a mouse. Monoclonal antibodies may then be harvested from
25 the ascites fluid or the blood. Contaminants may be removed from the antibodies by conventional techniques, such as chromatography, gel filtration, precipitation, and extraction. The polypeptides of this invention may be used in the purification process in, for example, an affinity chromatography step.

Monoclonal antibodies of the present invention may also be used as
30 therapeutic reagents, to diminish or eliminate prostate tumors. The antibodies may be

used on their own (for instance, to inhibit metastases) or coupled to one or more therapeutic agents. Suitable agents in this regard include radionuclides, differentiation inducers, drugs, toxins, and derivatives thereof. Preferred radionuclides include ⁹⁰Y, ¹²³I, ¹²⁵I, ¹³¹I, ¹⁸⁶Re, ¹⁸⁸Re, ²¹¹At, and ²¹²Bi. Preferred drugs include methotrexate, and 5 pyrimidine and purine analogs. Preferred differentiation inducers include phorbol esters and butyric acid. Preferred toxins include ricin, abrin, diphteria toxin, cholera toxin, gelonin, Pseudomonas exotoxin, Shigella toxin, and pokeweed antiviral protein.

A therapeutic agent may be coupled (e.g., covalently bonded) to a suitable monoclonal antibody either directly or indirectly (e.g., via a linker group). A 10 direct reaction between an agent and an antibody is possible when each possesses a substituent capable of reacting with the other. For example, a nucleophilic group, such as an amino or sulfhydryl group, on one may be capable of reacting with a carbonyl-containing group, such as an anhydride or an acid halide, or with an alkyl group containing a good leaving group (e.g., a halide) on the other.

15 Alternatively, it may be desirable to couple a therapeutic agent and an antibody via a linker group. A linker group can function as a spacer to distance an antibody from an agent in order to avoid interference with binding capabilities. A linker group can also serve to increase the chemical reactivity of a substituent on an agent or an antibody, and thus increase the coupling efficiency. An increase in 20 chemical reactivity may also facilitate the use of agents, or functional groups on agents, which otherwise would not be possible.

It will be evident to those skilled in the art that a variety of bifunctional or polyfunctional reagents, both homo- and hetero-functional (such as those described in the catalog of the Pierce Chemical Co., Rockford, IL), may be employed as the linker 25 group. Coupling may be effected, for example, through amino groups, carboxyl groups, sulfhydryl groups or oxidized carbohydrate residues. There are numerous references describing such methodology, e.g., U.S. Patent No. 4,671,958, to Rodwell et al.

Where a therapeutic agent is more potent when free from the antibody portion of the immunoconjugates of the present invention, it may be desirable to use a 30 linker group which is cleavable during or upon internalization into a cell. A number of

different cleavable linker groups have been described. The mechanisms for the intracellular release of an agent from these linker groups include cleavage by reduction of a disulfide bond (e.g., U.S. Patent No. 4,489,710, to Spitler), by irradiation of a photolabile bond (e.g., U.S. Patent No. 4,625,014, to Senter et al.), by hydrolysis of 5 derivatized amino acid side chains (e.g., U.S. Patent No. 4,638,045, to Kohn et al.), by serum complement-mediated hydrolysis (e.g., U.S. Patent No. 4,671,958, to Rodwell et al.), and acid-catalyzed hydrolysis (e.g., U.S. Patent No. 4,569,789, to Blattler et al.).

It may be desirable to couple more than one agent to an antibody. In one embodiment, multiple molecules of an agent are coupled to one antibody molecule. In 10 another embodiment, more than one type of agent may be coupled to one antibody. Regardless of the particular embodiment, immunoconjugates with more than one agent may be prepared in a variety of ways. For example, more than one agent may be coupled directly to an antibody molecule, or linkers which provide multiple sites for attachment can be used. Alternatively, a carrier can be used.

15 A carrier may bear the agents in a variety of ways, including covalent bonding either directly or via a linker group. Suitable carriers include proteins such as albumins (e.g., U.S. Patent No. 4,507,234, to Kato et al.), peptides and polysaccharides such as aminodextran (e.g., U.S. Patent No. 4,699,784, to Shih et al.). A carrier may also bear an agent by noncovalent bonding or by encapsulation, such as within a 20 liposome vesicle (e.g., U.S. Patent Nos. 4,429,008 and 4,873,088). Carriers specific for radionuclide agents include radiohalogenated small molecules and chelating compounds. For example, U.S. Patent No. 4,735,792 discloses representative radiohalogenated small molecules and their synthesis. A radionuclide chelate may be formed from chelating compounds that include those containing nitrogen and sulfur 25 atoms as the donor atoms for binding the metal, or metal oxide, radionuclide. For example, U.S. Patent No. 4,673,562, to Davison et al. discloses representative chelating compounds and their synthesis.

A variety of routes of administration for the antibodies and immunoconjugates may be used. Typically, administration will be intravenous, 30 intramuscular, subcutaneous or in the bed of a resected tumor. It will be evident that the

precise doses of the antibody/immunoconjugate will vary depending upon the antibody used, the antigen density on the tumor, and the rate of clearance of the antibody.

Diagnostic reagents of the present invention may also comprise DNA sequences encoding one or more of the above polypeptides, or one or more portions thereof. For example, at least two oligonucleotide primers may be employed in a polymerase chain reaction (PCR) based assay to amplify prostate tumor-specific cDNA derived from a biological sample, wherein at least one of the oligonucleotide primers is specific for a DNA molecule encoding a polypeptide of the present invention. The presence of the amplified cDNA is then detected using techniques well known in the art, such as gel electrophoresis. Similarly, oligonucleotide probes specific for a DNA molecule encoding a polypeptide of the present invention may be used in a hybridization assay to detect the presence of an inventive polypeptide in a biological sample.

As used herein, the term "oligonucleotide primer/probe specific for a DNA molecule" means an oligonucleotide sequence that has at least about 80% identity, preferably at least about 90% and more preferably at least about 95%, identity to the DNA molecule in question. Oligonucleotide primers and/or probes which may be usefully employed in the inventive diagnostic methods preferably have at least about 10-40 nucleotides. In a preferred embodiment, the oligonucleotide primers comprise at least about 10 contiguous nucleotides of a DNA molecule encoding one of the polypeptides disclosed herein. Preferably, oligonucleotide probes for use in the inventive diagnostic methods comprise at least about 15 contiguous oligonucleotides of a DNA molecule encoding one of the polypeptides disclosed herein. Techniques for both PCR based assays and hybridization assays are well known in the art (see, for example, Mullis *et al. Ibid*; Ehrlich, *Ibid*). Primers or probes may thus be used to detect prostate and/or prostate tumor sequences in biological samples, preferably blood, semen or prostate and/or prostate tumor tissue.

The following Examples are offered by way of illustration and not by way of limitation.

EXAMPLES

Example 15 A. Isolation of Polypeptides from LnCap.fgc using human prostatitis sera

Representative polypeptides of the present invention were isolated by screening a human prostate cancer cell line with human prostatitis sera as follows. A human prostate adenocarcinoma cDNA expression library was constructed by reverse transcriptase synthesis from mRNA purified from the human prostate adenocarcinoma cell line LnCap.fgc (ATCC No. 1740-CRL), followed by insertion of the resulting cDNA clones in Lambda ZAP II (Stratagene, La Jolla, CA).

Human prostatitis serum was obtained from a patient diagnosed with autoimmune prostatitis following treatment of bladder carcinoma by administration of BCG. This serum was used to screen the LnCap cDNA library as described in Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratories, Cold Spring Harbor, NY, 1989. Specifically, LB plates were overlaid with approximately 10^4 pfu of the LnCap cDNA library and incubated at 42°C for 4 hours prior to obtaining a first plaque lift on isopropylthio-beta-galactoside (IPTG) impregnated nitrocellulose filters. The plates were then incubated for an additional 5 hours at 42°C and a second plaque lift was prepared by incubation overnight at 37°C. The filters were washed three times with PBS-T, blocked for 1 hours with PBS (containing 1% Tween 20TM) and again washed three times with PBS-T, prior to incubation with human prostatitis sera at a dilution of 1:200 with agitation overnight. The filters were then washed three times with PBS-T and incubated with ¹²⁵I-labeled Protein A (1 µl/15 ml PBS-T) for 1 hour with agitation. Filters were exposed to film for variable times, ranging from 16 hours to 7 days. Plaques giving signals on duplicate lifts were re-plated on LB plates. Resulting plaques were lifted with duplicate filters and these filters were treated as above. The filters were incubated with human prostatitis sera (1:200 dilution) at 4°C with agitation overnight. Positive plaques were

visualized with ^{125}I -Protein A as described above with the filters being exposed to film for variable times, ranging from 16 hours to 11 days. *In vivo* excision of positive human prostatitis antigen cDNA clones was performed according to the manufacturer's protocol.

5

B. Characterization of Polypeptides

DNA sequence for positive clones was obtained using forward and reverse primers on an Applied Biosystems Inc. Automated Sequence Model 373A (Foster City, CA). The cDNA sequences encoding the isolated polypeptides, 10 hereinafter referred to as HPA8, HPA13, HPA15 - HPA17, HPA20, HPA25, HPA28, HPA29, HPA32 - HPA38 and HPA41 are presented in SEQ ID Nos. 32 and 33, 34 and 35, 36, 9 and 10, 11, 12, 13 and 14, 15, 37 and 38, 16, 39, 22 and 23, 17 and 18, 19, 24, 40 and 41, 42 and 43, respectively. The 3' sequences of HPA16 and HPA20 are identical. HPA13, HPA16, HPA20, HPA29 and HPA33 are believed to be overlapping 15 clones with novel 5' end points. Two of the positive clones were determined to be identical to HPA15. Also, HPA15, HPA34 and HPA37 were found to be overlapping clones. The expected N-terminal amino acid sequences of the isolated polypeptides HPA16, HPA17, HPA20, HPA25, HPA28, HPA32, HPA35, HPA36, HPA34, HPA37, HPA8, HPA13, HPA15, HPA29, HPA33, HPA38 and HPA41, based on the determined 20 cDNA sequences in frame with the N-terminal portion of β -galactosidase (*lacZ*) are presented in SEQ ID Nos. 1-8, 20, 21 and 25-31, respectively.

The determined cDNA and expected amino acid sequences for the isolated polypeptides were compared to known sequences in the gene bank using the EMBL and GenBank (Release 91) databases, and also the DNA STAR system. The 25 DNA STAR system is a combination of the Swiss, PIR databases along with translated protein sequences (Release 91). No significant homologies to HPA17, HPA25, HPA28, HPA32, HPA35 and HPA36 were found.

The determined cDNA sequence for HPA8 was found to have approximately 100% identity with the human proto-oncogene BMI-1 (Alkema, M.J. 30 et al., *Hum. Mol. Gen.* 2:1597-1603, 1993). Search of the DNA database with 5' and 3'

cDNA sequence encoding HPA13 revealed 100% identity with a known cDNA sequence from a human immature myeloid cell line (GenBank Acc. No. D63880). Search of the protein database with the deduced amino acid sequence for HPA13 revealed 100% identity with the open reading frame encoded by the same human cDNA sequence. Search of the protein database with the expected amino acid sequence for HPA15, revealed high homology (60% identity) with a *Saccharomyces cerevisiae* predicted open reading frame (Swiss/PIR Acc. No. S46677), and 100% identity with a human protein from pituitary gland modulating intestinal fluid secretion (Lonnroth, I., *J. Biol. Chem.* 35:20615-20620, 1995). The deduced amino acid sequence for HPA38 was found to have 100% identity with human heat shock factor protein 2 (Schuetz, T. J. et al., *Proc. Natl. Acad. Sci. USA* 88:6911-6915, 1991). Search of the DNA database with the 5' DNA sequence for HPA41 and search of the protein database with the deduced amino acid sequence revealed 100% identity with a human LIM protein (Rearden, A., *Biochem. Biophys. Res. Commun.* 201:1124-1131, 1994). To the best of the inventors' knowledge, except for LIM protein, none of the inventive polypeptides have been previously shown to be present in human prostate.

Positive phagemid viral particles were used to infect *E. coli* XL-1 Blue MRF', as described in Sambrook et al., *supra*. Induction of recombinant protein was accomplished by the addition of IPTG. Induced and uninduced lysates were run in duplicate on SDS-PAGE and transferred to nitrocellulose filters. Filters were reacted with human prostatitis sera (1:200 dilution) and a rabbit sera (1:200 or 1:250 dilution) reactive with the N-terminal 4 Kd portion of lacZ. Sera incubations were performed for 2 hours at room temperature. Bound antibody was detected by addition of ¹²⁵I-labeled Protein A and subsequent exposure to film for variable times ranging from 16 hours to 25 11 days. The results of the immunoblots are summarized in Table I, wherein (+) indicates a positive reaction and (-) indicates no reaction.

TABLE I

	<u>Antigen</u>	<u>Human Prostatitis Sera</u>	<u>Anti-lacZ Sera</u>	<u>Protein Mass/Kd</u>
5	HPA8	(-)	(-)	
	HPA13	(+)	(+)	
	HPA15	(+)	(+)	50
	HPA16	(+)	(+)	40
10	HPA17	(+)	(-)	40
	HPA20	(+)	(+)	38
	HPA25	(-)	(+)	32
	HPA28	(-)	(-)	
	HPA29	(+)	(+)	
15	HPA32	(-)	(-)	
	HPA33	(+)	(+)	
	HPA34	not tested	(+)	50
	HPA35	(-)	(-)	
	HPA36	(-)	(-)	
20	HPA37	not tested	(+)	50
	HPA38	(-)	(-)	
	HPA41	not tested	(+)	

Positive reaction of the recombinant human prostatitis antigens with both the human prostatitis sera and anti-lacZ sera indicate that reactivity of the human prostatitis sera is directed towards the fusion protein. Cloned antigens showing reactivity to the human prostatitis sera but not to anti-lacZ sera indicate that the reactive protein is likely initiating within the clone. Antigens reactive with the anti-lacZ sera but not with the human prostatitis sera may be the result of the human prostatitis sera recognizing conformational epitopes, or the antigen-antibody binding kinetics may be such that the 2 hour sera exposure in the immunoblot is not sufficient. Antigens not

reactive with either sera are not being expressed in *E. coli*, and reactive epitopes may be within the fusion protein or within an internal open reading frame. Due to the instability of recombinant antigens from HPA13, HPA29 and HPA33, it was not possible to determine the size of the recombinant antigens.

5 The expression of representative human prostatitis antigens was investigated by RT-PCR in four different human cell lines (including two metastatic prostate tumor lines LNCaP and DU145), normal prostate, breast, colon, kidney, stomach, lung and skeletal muscle tissue, nine different prostate tumor samples and three different breast tumor samples. The results of these studies are shown in Table II.

Table II
Analysis of HPA clone mRNA expression by RT-PCR in human cell lines, normal tissues and tumors

S	Clone	Breast Tumors (n=3)											
		LNCaP	DU145	MCF-12A	HBL-100	Prostate	Breast	Colon	Kidney	Stomach	Lung		
10	hpa-17	+	++	+	+	-	+	-	-	+	+		
	hpa-20	+++	++++	NT	+	NT	NT	-	NT	+	NT		
	hpa-28	+	+++	+	+	-	+	+	-	+	+		
15	Prostate Tumors (n=9)										Breast Tumors (n=3)		
	Clone	Tumor 1	Tumor 2	Tumor 3	Tumor 4	Tumor 5	Tumor 6	Tumor 7	Tumor 8	Tumor 9	Tumor 1	Tumor 2	Tumor
	hpa-17 ++	+	+	+	-	+	+	+	-	-	+	+	++
20	hpa-20	+	+	NT	NT	NT	NT	NT	NT	NT	+	+	++
	hpa-28	+	+	+	-	+	+	++	+	-	++	+++	++

mRNA expression of representative antigens in LNCaP and normal prostate, kidney, liver, stomach, lung and pancreas was also investigated by RNase protection. The results of these studies are provided in Table III.

5

Table III

**Analysis of HPA clone mRNA expression by RNase protection in LNCaP and
normal human tissues**

<u>Clone</u>	<u>LNCaP</u>	<u>Prostate</u>	<u>Kidney</u>	<u>Liver</u>	<u>Stomach</u>	<u>Lung</u>	<u>Pancreas</u>
hpa-15	+	-	++	++	+	-	++
hpa-20	+++++	+	+	+	+	NT	NT
hpa-25	+	+	+	+	++	++	NT
hpa-32	NT	++	+	+	NT	++	NT
hpa-35	++	+++	NT	+	+	+++	+
hpa-36	+	+	NT	NT	+	+	+

10

Example 2A. Isolation and Characterization of Rat Steroid Binding Protein

Immune sera was obtained from rats immunized with rat prostate extract to generate antibodies to self prostate antigens. Specifically, rats were prebled to obtain 15 control sera prior to being immunized with a detergent extract of rat prostate (in PBS containing 0.1% Triton) in Freunds complete adjuvant. A boost of incomplete Freunds adjuvant was given 3 weeks after the initial immunization and sera was harvested at 6 weeks.

The sera thus obtained was subjected to ECL Western blot analysis 20 (Amersham International, Arlington Heights, Ill) using the manufacturer's protocol and a rat prostate protein was identified, as shown in Fig. 1. After reduction, SDS-PAGE revealed a broad silver staining band migrating at 7 kD. Without reduction, a strong band was seen at 24 kD (Fig. 2). This protein was purified by ion exchange

chromatography and subjected to gel electrophoresis under reduced conditions. Three bands were seen, indicating the presence of three chains within the protein: a 6-8 kD chain (C1), a 8-10 kD chain (C2) and a 10-12 kD chain (C3). The protein was further purified by reverse phase HPLC on a DeltaTM C18 300 A° 5 μm column, column size 5 3.9 x 300 mm (Waters-Millipore, Milford, MA). The sample containing 100 μg of protein was dissolved in 0.1% trifluoroacetic acid (TFA), pH 1.9 and polypeptides were eluted with a linear gradient of acetonitrile (0-60%) in 0.1% TFA pH 1.9 at a flow rate of 0.5 mL/min for 1 hour. The eluent was monitored at 214 nm. Two peaks were obtained, a C1-C3 dimer and a C2-C3 dimer. The amino terminus of the C2 chain was 10 found to be blocked. The C1 and C3 chains were sequenced on a Perkin Elmer/Applied Biosystems Inc. Procise Model 494 protein sequencer and found to have the following amino terminal sequences (Seq. ID Nos. 44 and 45, respectively).

(a) Ser-Gln-Ile-Cys-Glu-Leu-Val-Ala-His-Glu-Thr-Ile-Ser-Phe-Leu; and
(b) Xaa-Xaa-Xaa-Xaa-Xaa-Ser-Ile-Leu-asp-Glu-Val-Ile-Arg-Gly-Thr,
15 wherein Xaa may be any amino acid.

These sequences were compared to known sequences in the gene bank using the databases discussed in Example 1 and were found to be identical to rat steroid binding protein, also known as estramustine-binding protein (EMBP) (Forsgren, B. et al., *Prog. Clin. Biol. Res.* 75A:391-407, 1981; Forsgren, B. et al., *Proc. Natl. Acad. Sci. USA* 76:3149-53, 1979). This protein is a major secreted protein in rat seminal fluid and has been shown to bind steroid, cholesterol and proline rich proteins. EMBP has been shown to bind estramustine and estromustine, the active metabolites of estramustine phosphate. Estramustine phosphate has been found to be clinically useful in treating advanced prostate cancer in patients who do not respond to standard 20 hormone ablation therapy (see, for example, Van Poppel, H. et al., *Prog. Clin. Biol. Res.* 370:323-41, 1991).

B. Isolation of putative human homologue to rat steroid binding protein

Purified rat steroid binding protein was obtained from freshly excised rat 30 prostate and used to subcutaneously immunize a New Zealand white virgin female

rabbit (150 µg purified rat steroid binding protein in 1 ml of PBS and 1 ml of incomplete Freund's adjuvant containing 100 µg of muramyl dipeptide (adjuvant peptide, Calbiochem, La Jolla, CA). Six weeks later the rabbit was boosted subcutaneously with the same protein dose in incomplete Freund's adjuvant. Finally, 5 the rabbit was boosted intravenously two weeks later with 100 µg protein in PBS and the sera harvested two weeks after the final immunization.

The resulting rabbit antisera was used to screen the LnCap.fgc cell line without success. The rabbit antisera was subsequently used to screen human seminal fluid anion exchange chromatography pools using the protocol detailed below in 10 Example 3. This analysis indicated an approximately 18-22 kD cross-reactive protein. The seminal fluid fraction of interest (Fraction 1) was separated into individual components by SDS-PAGE under non-reducing conditions, blotted onto a PVDF membrane, excised and digested with CNBr in 70% formic acid. The resulting CNBr fragments were resolved on a tricine gel system, again electroblotted to PVDF and 15 excised. The sequence for one peptide was determined as follows:

Val-Val-Lys-Thr-Tyr-Leu-Ile-Ser-Ser-Ile-Pro-Leu-Gln-Gly-Ala-Phe-
Asn-Tyr-Lys-Tyr-Thr-Ala (SEQ. ID No. 46).

This sequence was compared to known sequences in the gene bank using the databases identified above and was unexpectedly found to be identical to gross 20 cystic disease fluid protein, a protein whose expression was previously found to correlate with the presence of metastatic breast cancer (Murphy, L.C. et al., *J. Biol. Chem.* 262:15236-15241, 1987). To the best of the inventors' knowledge, this protein has not been previously identified in male tissues.

The ability of Fraction 1 as described above, to bind to steroid was 25 investigated as follows. Purified rat steroid binding protein (RSBP) and fraction 1 were subjected to SDS-PAGE and transferred onto nitrocellulose filters. Specifically, 1.5 µg of RSBP/gel lane and 4 µg of fraction 1/gel lane were electrophoresed in parallel on a 4-20% gradient Laemmli gel (BioRad), then electrophoretically transferred to nitrocellulose. After protein transfer, the nitrocellulose was blocked for 1 hour at 30 room temperature in 1% Tween 20 in PBS, rinsed three times for 10 min each in 10 ml

0.1% Tween 20 in PBS plus 0.5 M NaCl, then probed with either 1) 0.87 μ M progesterone conjugated to horseradish peroxidase (HRP, Sigma) diluted in the rinse buffer; 2) 0.87 μ M progesterone HRP with 200 μ M estramustine; or 3) 0.87 μ M progesterone HRP plus 400 μ M unlabelled progesterone and 200 μ M estramustine.

- 5 Each reaction mixture was incubated for 1 hour at room temperature and washed three times for 10 min each with 0.1% Tween 20 , PBS, and 0.5 M NaCl. The blots were then developed (ECL system, Amersham) to reveal progesterone HRP binding proteins that are also capable of binding estramustine.

With both rat steroid binding protein and Fraction 1, three bands were obtained that bound HRP-progesterone and that were competed out with unlabelled progesterone and estramustine (Fig. 3). These results indicate that the three bands isolated from human seminal fluid as described above bind hormone and correspond in number of polypeptides to the chains C1, C2 and C3 of rat steroid binding protein, although slightly bigger in size, either due to primary sequence or secondary post-translational modifications.

This putative homologue of rat steroid binding protein was also identified in a subsequent screen of human seminal fluid using the rabbit antisera detailed above. Specifically a hydrophobic 22kD/65kD aggregate protein was obtained which, following CNBr digestion of the 22kD band, provided a peptide having the following sequence:

Val-Val-Lys-Thr-Tyr-Leu-Ile-Ser-Ser-Ile-Pro-Leu-Gln-Ala-Phe-Asn-Tyr-Lys-Tyr-Thr-Ala (SEQ. ID No. 47).

This peptide was found to correspond to residues 67 through 87 of gross cystic disease fluid protein and was identified again utilizing human autoimmune prostatitis sera as discussed below in Example 4.

Example 3Isolation and Characterization of Polypeptides Isolated from LnCap.fgcUsing Rat Prostatitis Sera

5 A LnCap.fgc cell pellet was homogenized (10 gm cell pellet in 10 ml) by resuspension in PBS, 1% NP-40 and 60 µg/ml phenylmethylsulfonyl fluoride (PMSF) (Sigma, St. Louis, MO) then 10 strokes in a Dounce homogenizer. This was followed by a 30 second probe sonication and another 10 strokes in the Dounce homogenizer. The resulting slurry was centrifuged at 10,000 x G, and the supernatant filtered with a
10 0.45 µM filter (Amicon, Beverly, MA) then applied to a BioRad (Hercules, CA) Macro-Prep Q-20 anion exchange resin. Proteins were eluted with a 70 minute 0 to 0.8 M NaCl gradient in 20 mM tris pH 7.5 at a flow rate of 8 ml/min. Fractions were cooled, concentrated with 10 kD MWCO centriprep concentrators (Amicon) and stored at -20°C in the presence of 60 µg/ml PMSF. The ion exchange pools were then examined
15 by electrophoresis on 4-20% tris glycine Ready-Gels (BioRad) and subsequent transfer to nitrocellulose filters. Ion exchange pools of interest were identified by ECL (Amersham International) Western analysis, using the rat sera described above in Example 3A. This analysis indicated an approximately 65 kD protein eluting at 0.08 to 0.13 M NaCl. The rat sera reactive ion exchange pool was subjected to HPLC and
20 subsequent Western analysis to identify the protein fraction of interest. This protein was then digested for 24 hours at 25°C in 70% formic acid saturated with CNBr to cleave at methionine residues.

The resulting CNBr fragments were purified by microbore HPLC using a Vydac C18 column (Hesperia, CA), column size 1x150 mM in a Perkin Elmer/Applied
25 Biosystems Inc. (Foster City, CA) Division Model 172 HPLC. Fractions were eluted from the column with a gradient of 0 to 60% of acetonitrile at a flow rate of 40 µl per minute. The eluent was monitored at 214 nm. The resulting fractions were loaded directly onto a Perkin Elmer/Applied Biosystems Inc. Procise 494 protein sequencer and sequenced using standard Edman chemistry from the amino terminal end. Two
30 different peptides having the following sequences were obtained:

- (a) Xaa-Ala-Lys-Lys-Phe-Leu-Asp-Ala-Glu-His-Lys-Leu-Asn-Phe-Ala (SEQ. ID No. 48); and
- (b) Xaa-Xaa-Xaa-Lys-Ile-Lys-Lys-Phe-Ile-Gln-Glu-Asn-Ile-Phe-Gly,
- 5 wherein Xaa may be any amino acid (SEQ ID No. 49).

These sequences were compared to known sequences in the gene bank using databases identified above, and identified as residues 286 through 300 and 228 through 242, respectively, of probable protein disulfide isomerase ER-60 precursor, hereinafter referred to as ER-60 (Bado, R. J. et al., *Endocrinology* 123:1264-1273, 10 1988). This antigen is also known as phospholipase C-alpha (see PCT WO 95/08624). Residues 285 and 227 of ER-60 are methionines, consistent with the above sequences being cyanogen bromide fractions.

ER-60 is a resident endoplasmic protein with multiple biological activities, including disulfide isomerase and restricted cysteine protease activity. In 15 particular, ER-60 has been shown to preferentially degrade calnexin, a protein involved in presentation of antigens via the Class I major histocompatibility complex, or MHC, pathway. ER-60 and a related family member, ER-72, have been shown to be over-expressed in colon cancer, with truncated forms of ER-60 exhibiting increased enzymatic activity (Egea, G. et al., *J. Cell. Sci. (England)* 105:819-30, 1993). However, 20 to the best of the inventors' knowledge, this polypeptide has not been previously shown to be present or overexpressed in human prostate. Recently, ER-60 gene expression has been correlated with induction of contact inhibition of cell proliferation (Greene, J.J. et al., *Cell. Mol. Biol.* 41:473-80, 1995). Thus, if ER-60 is also truncated and non-functional in prostate cancer, as it is in colon cancer, the resultant loss of contact 25 inhibition would lead to neoplastic transformation and tumor progression.

Example 4Isolation and Characterization of Polypeptides Isolated from LnCaP.fgcUsing Human Prostatitis Sera

5 The human prostatitis sera described above in Example 1 was used to screen the LnCaP.fgc cell line using the ion exchange techniques described above in Example 3. Reactive ion exchange pools were purified by reverse phase HPLC as described previously and the polypeptides shown in SEQ ID Nos. 50-51 were isolated utilizing cross-reactivity with said antisera as the selection criteria. Comparison of
10 these sequences with known sequences in the gene bank using the databases described above revealed the homologies shown in Table II. However, none of these polypeptides have been previously associated with human prostate.

TABLE IV

	<u>SEQ ID No.</u>	<u>Database Search Identification</u>
15	53	glyceraldehyde-3-phosphate-dehydrogenase
	54	alpha-human fructose biphosphate aldolase
20	55	calreticulin
	56	calreticulin
	57	malate dehydrogenase
	58	cystic disease fluid protein
	59	cystic disease fluid protein

Example 5Isolation and Characterization of Polypeptides from Human Seminal Fluid

Polypeptides from human seminal fluid were purified to homogeneity by
5 anion exchange chromatography. Specifically, seminal fluid samples were diluted 1 to
10 with 0.1 mM Bis-Tris propane buffer pH 7 prior to loading on the column. The
polypeptides were fractionated into pools utilizing gel profusion chromatography on a
Poros (Perseptive Biosystems) 146 II Q/M anion exchange column 4.6 mm x 100 mm
equilibrated in 0.01 mM Bis-Tris propane buffer pH 7.5. Proteins were eluted with a
10 linear 0-0.5 M NaCl gradient in the above buffer. The column eluent was monitored at
a wavelength of 220 nm. Individual fractions were further purified by reverse phase
HPLC on a Vydac (Hesperia, CA) C18 column.

The resulting fractions were sequenced as described above in Example 3.
A peptide having the following N-terminal sequence was obtained:

15 (c) Met-Asp-Ile-Pro-Gln-Thr-Lys-Gln-Asp-Leu-Glu-Leu-Pro-Lys-Leu
(SEQ ID NO:57).

Comparison of this sequence with those of known sequences in the gene bank as
described above revealed 100% identity with human placental protein 14 (PP14).

20

Example 6Synthesis of Polypeptides

Polypeptides may be synthesized on an Applied Biosystems 430A
25 peptide synthesizer using FMOC chemistry with HPTU (O-Benzotriazole-N,N,N',N'-
tetramethyluronium hexafluorophosphate) activation. A Gly-Cys-Gly sequence may be
attached to the amino terminus of the peptide to provide a method of conjugation,
binding to an immobilized surface, or labeling of the peptide. Cleavage of the peptides
from the solid support may be carried out using the following cleavage mixture:
30 trifluoroacetic acid:ethanedithiol:thioanisole:water:phenol (40:1:2:2:3). After cleaving

for 2 hours, the peptides may be precipitated in cold methyl-t-butyl-ether. The peptide pellets may then be dissolved in water containing 0.1% trifluoroacetic acid (TFA) and lyophilized prior to purification by C18 reverse phase HPLC. A gradient of 0%-60% acetonitrile (containing 0.1% TFA) in water (containing 0.1% TFA) may be used to
5 elute the peptides. Following lyophilization of the pure fractions, the peptides may be characterized using electrospray or other types of mass spectrometry and by amino acid analysis.

From the foregoing, it will be appreciated that, although specific
10 embodiments of the invention have been described herein for the purposes of illustration, various modifications may be made without deviating from the spirit and scope of the invention.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: Corixa Corporation
- (ii) TITLE OF INVENTION: COMPOUNDS AND METHODS FOR IMMUNOTHERAPY AND IMMUNODIAGNOSIS OF PROSTATE CANCER

(iii) NUMBER OF SEQUENCES: 57

(iv) CORRESPONDENCE ADDRESS:

- (A) ADDRESSEE: SEED and BERRY LLP
- (B) STREET: 6300 Columbia Center, 701 Fifth Avenue
- (C) CITY: Seattle
- (D) STATE: Washington
- (E) COUNTRY: USA
- (F) ZIP: 98104-7092

(v) COMPUTER READABLE FORM:

- (A) MEDIUM TYPE: Floppy disk
- (B) COMPUTER: IBM PC compatible
- (C) OPERATING SYSTEM: PC-DOS/MS-DOS
- (D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:

- (A) APPLICATION NUMBER:
- (B) FILING DATE: 14-MAR-1997
- (C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:

- (A) NAME: Maki, David J.
- (B) REGISTRATION NUMBER: 31,392
- (C) REFERENCE/DOCKET NUMBER: 210121.424PC

(ix) TELECOMMUNICATION INFORMATION:

- (A) TELEPHONE: (206) 622-4900
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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 89 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Ala Arg Ala Ser Val Met Leu Leu Gly Met Met Ala Arg Gly Lys Pro
1 5 10 15

Glu Ile Val Gly Ser Asn Leu Asp Thr Leu Met Ser Ile Gly Leu Asp
20 25 30

Glu Lys Phe Pro Gln Asp Tyr Arg Leu Ala Gln Gln Val Cys His Ala
35 40 45

Ile Ala Asn Ile Ser Asp Arg Arg Lys Pro Ser Leu Gly Lys Arg His
 50 55 60

Pro Pro Phe Arg Leu Pro Gln Glu His Arg Leu Phe Glu Arg Leu Arg
 65 70 75 80

Glu Thr Val Thr Lys Gly Phe Val His
 85

(2) INFORMATION FOR SEQ ID NO:2:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 89 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS:
 - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Ala Arg Gly Arg Phe Gly Arg Leu Gly Val Gly Gly Glu Pro His Pro
 1 5 10 15

Arg Arg Asn Pro Ala Leu Pro Thr Glu Leu Ala Glu Leu Thr Pro Gln
 20 25 30

Val Arg Arg Ala Ala Xaa Lys Thr Gln Arg Ser Gln Val Lys Pro Arg
 35 40 45

His Arg Arg Gly Trp Pro Pro Thr Val Pro Leu Ala Gly Arg Leu Glu
 50 55 60

Glu Leu Lys Thr Pro Arg Ser Pro Arg Pro Pro Glu Gln Gly Leu Asp
 65 70 75 80

Pro Ser Pro Cys Ser Leu Pro Ser Pro
 85

(2) INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 858 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS:
 - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Gln Glu Ser Glu Pro Phe Ser His Ile Asp Pro Glu Glu Ser Glu Glu
 1 5 10 15

Thr Arg Leu Leu Asn Ile Leu Gly Leu Ile Phe Lys Gly Pro Ala Ala
 20 25 30

Ser Thr Gln Glu Lys Asn Pro Arg Glu Ser Thr Gly Asn Met Val Thr
 35 40 45
 Gly Gln Thr Val Cys Lys Asn Lys Pro Asn Met Ser Asp Pro Glu Glu
 50 55 60
 Ser Arg Gly Asn Asp Glu Leu Val Lys Gln Glu Met Leu Val Gln Tyr
 65 70 75 80
 Leu Gln Asp Ala Tyr Ser Phe Ser Arg Lys Ile Thr Glu Ala Ile Gly
 85 90 95
 Ile Ile Ser Lys Met Met Tyr Glu Asn Thr Thr Thr Val Val Gln Glu
 100 105 110
 Val Ile Glu Xaa Phe Val Met Val Phe Gln Phe Gly Val Pro Gln Ala
 115 120 125
 Leu Phe Gly Val Arg Arg Met Leu Pro Leu Ile Trp Ser Lys Glu Pro
 130 135 140
 Gly Val Arg Glu Ala Val Leu Asn Ala Tyr Arg Gln Leu Tyr Leu Asn
 145 150 155 160
 Pro Lys Gly Asp Ser Ala Arg Ala Lys Ala Gln Ala Leu Ile Gln Asn
 165 170 175
 Leu Ser Leu Leu Leu Val Asp Ala Ser Val Gly Thr Ile Gln Cys Leu
 180 185 190
 Glu Glu Ile Leu Cys Glu Phe Val Gln Lys Asp Glu Leu Lys Pro Ala
 195 200 205
 Val Thr His Leu Leu Trp Glu Arg Ala Thr Glu Lys Val Ala Cys Cys
 210 215 220
 Pro Leu Glu Arg Cys Ser Ser Val Met Leu Leu Gly Met Met Ala Arg
 225 230 235 240
 Arg Lys Pro Glu Ile Val Gly Ser Asn Leu Asp Thr Leu Met Ser Ile
 245 250 255
 Gly Leu Asp Glu Lys Phe Pro Gln Asp Tyr Arg Leu Ala Gln Gln Val
 260 265 270
 Cys His Ala Ile Ala Asn Ile Ser Asp Arg Arg Lys Pro Ser Leu Gly
 275 280 285
 Lys Arg His Pro Pro Phe Arg Leu Pro Gln Glu His Arg Leu Phe Glu
 290 295 300
 Arg Leu Arg Glu Thr Val Thr Lys Gly Phe Val His Pro Asp Pro Leu
 305 310 315 320
 Trp Ile Pro Phe Lys Glu Val Ala Val Thr Leu Ile Tyr Gln Leu Ala
 325 330 335
 Glu Gly Pro Glu Val Ile Cys Ala Gln Ile Leu Gln Gly Cys Ala Lys
 340 345 350
 Gln Ala Leu Glu Lys Leu Glu Glu Lys Arg Thr Ser Gln Glu Asp Pro
 355 360 365

Lys Glu Ser Pro Ala Met Leu Pro Thr Phe Leu Leu Met Asn Leu Leu
 370 375 380
 Ser Leu Ala Gly Asp Val Ala Leu Gln Gln Leu Val His Leu Glu Gln
 385 390 395 400
 Ala Val Ser Gly Glu Leu Cys Arg Arg Val Leu Arg Glu Glu Gln
 405 410 415
 Glu His Lys Thr Lys Asp Pro Lys Glu Lys Asn Thr Ser Ser Glu Thr
 420 425 430
 Thr Met Glu Glu Glu Leu Gly Leu Val Gly Ala Thr Ala Asp Asp Thr
 435 440 445
 Glu Ala Glu Leu Ile Arg Gly Ile Cys Glu Met Glu Leu Leu Asp Gly
 450 455 460
 Lys Gln Thr Leu Ala Ala Phe Val Pro Leu Leu Leu Lys Val Cys Asn
 465 470 475 480
 Asn Pro Gly Leu Tyr Ser Asn Pro Asp Leu Ser Ala Ala Ala Ser Leu
 485 490 495
 Ala Leu Gly Lys Phe Cys Met Ile Ser Ala Thr Phe Cys Asp Ser Gln
 500 505 510
 Leu Arg Leu Leu Phe Thr Met Leu Glu Lys Ser Pro Leu Pro Ile Val
 515 520 525
 Arg Ser Asn Leu Met Val Ala Thr Gly Asp Leu Ala Ile Arg Phe Pro
 530 535 540
 Asn Leu Val Asp Pro Trp Thr Pro His Leu Tyr Ala Arg Leu Arg Asp
 545 550 555 560
 Pro Ala Gln Gln Val Arg Lys Thr Ala Gly Leu Val Met Thr His Leu
 565 570 575
 Ile Leu Lys Asp Met Val Lys Val Lys Gly Gln Val Ser Glu Met Ala
 580 585 590
 Val Leu Leu Ile Asp Pro Glu Pro Gln Ile Ala Ala Leu Ala Lys Asn
 595 600 605
 Phe Phe Asn Glu Leu Ser His Lys Gly Asn Ala Ile Tyr Asn Leu Leu
 610 615 620
 Pro Asp Ile Ile Ser Arg Leu Ser Asp Pro Glu Leu Gly Val Glu Glu
 625 630 635 640
 Glu Pro Phe His Thr Ile Met Lys Gln Leu Leu Ser Tyr Ile Thr Lys
 645 650 655
 Asp Lys Gln Thr Glu Ser Leu Val Glu Lys Leu Cys Gln Arg Phe Arg
 660 665 670
 Thr Ser Arg Thr Glu Arg Gln Arg Asp Leu Ala Tyr Cys Val Ser
 675 680 685
 Gln Leu Pro Leu Thr Glu Arg Gly Leu Arg Lys Met Leu Asp Asn Phe

690	695	700
Asp Cys Phe Gly Asp Lys Leu Ser Asp Glu Ser Ile Phe Ser Ala Phe		
705	710	715
Leu Ser Val Val Gly Lys Leu Arg Arg Gly Ala Lys Pro Glu Gly Lys		
725	730	735
Ala Ile Ile Asp Glu Phe Glu Gln Lys Leu Arg Ala Cys His Thr Arg		
740	745	750
Gly Leu Asp Gly Ile Lys Glu Leu Glu Ile Gly Gln Ala Gly Ser Gln		
755	760	765
Arg Ala Pro Ser Ala Lys Lys Pro Ser Thr Gly Ser Arg Tyr Gln Pro		
770	775	780
Leu Ala Ser Thr Ala Ser Asp Asn Asp Phe Val Thr Pro Glu Pro Arg		
785	790	795
Arg Thr Thr Arg Arg His Pro Asn Thr Gln Gln Arg Ala Ser Lys Lys		
805	810	815
Lys Pro Lys Val Val Phe Ser Ser Asp Glu Ser Ser Glu Glu Asp Leu		
820	825	830
Ser Ala Glu Met Thr Glu Asp Glu Thr Pro Lys Lys Thr Thr Pro Ile		
835	840	845
Leu Arg Ala Ser Ala Arg Arg His Arg Ser		
850	855	

(2) INFORMATION FOR SEQ ID NO:4:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 127 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS:
 - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Ala Arg Asp Arg Leu Val Ala Ser Lys Thr Asp Gly Lys Ile Val Gln		
1	5	10
Tyr Glu Cys Glu Gly Asp Thr Cys Gln Glu Glu Lys Ile Asp Ala Leu		
20	25	30
Gln Leu Glu Tyr Ser Tyr Leu Leu Thr Ser Gln Leu Glu Ser Gln Arg		
35	40	45
Ile Tyr Trp Glu Asn Lys Ile Val Arg Ile Glu Lys Asp Thr Ala Glu		
50	55	60
Glu Ile Asn Asn Met Lys Thr Lys Phe Lys Glu Thr Ile Xaa Xaa Cys		
65	70	75
Asp Asn Leu Glu His Xaa Leu Asn Asp Leu Leu Lys Glu Lys Gln Ser		

85	90	95
Val Glu Arg Lys Cys Thr Gln Leu Asn Thr Lys Val Ala Lys Leu Thr		
100	105	110
Asn Glu Leu Lys Glu Gln Glu Met Asn Lys Cys Leu Arg Ala		
115	120	125

(2) INFORMATION FOR SEQ ID NO:5:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 43 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS:
 - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Ala Arg Ala Glu Val Gln Arg Trp Arg Arg Leu Val Ala Gly Arg Arg			
1	5	10	15
Arg Ala Gly Gly Asp Gly Gly Asn Ser Gly Ser Cys Ser Arg Trp Gly			
20	25	30	
Gly Phe Thr Ser Tyr Pro Trp Asp Arg Glu Ile			
35	40		

(2) INFORMATION FOR SEQ ID NO:6:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 751 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS:
 - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Pro Ala Glu Ala His Ser Asp Ser Leu Ile Asp Thr Phe Pro Glu Cys			
1	5	10	15
Ser Thr Glu Gly Phe Ser Ser Asp Ser Asp Leu Val Ser Leu Thr Val			
20	25	30	
Asp Val Asp Ser Leu Ala Glu Leu Asp Asp Gly Met Ala Ser Asn Gln			
35	40	45	
Asn Ser Pro Ile Arg Thr Phe Gly Leu Asn Leu Ser Ser Asp Ser Ser			
50	55	60	
Ala Leu Gly Ala Val Ala Ser Asp Ser Glu Gln Ser Lys Thr Glu Glu			
65	70	75	80
Glu Arg Glu Ser Arg Ser Leu Phe Pro Gly Ser Leu Lys Pro Lys Leu			
85	90	95	

Gly Lys Arg Asp Tyr Leu Glu Lys Ala Gly Glu Leu Ile Lys Leu Ala
 100 105 110

Leu Lys Lys Glu Glu Asp Asp Tyr Glu Ala Ala Ser Asp Phe Tyr
 115 120 125

Arg Lys Gly Val Asp Leu Leu Glu Gly Val Gln Gly Glu Ser Ser
 130 135 140

Pro Thr Arg Arg Glu Ala Val Lys Arg Arg Thr Ala Glu Tyr Leu Met
 145 150 155 160

Arg Ala Glu Ser Ile Ser Ser Leu Tyr Gly Lys Pro Gln Leu Asp Asp
 165 170 175

Val Ser Gln Pro Pro Gly Ser Leu Ser Ser Arg Pro Leu Trp Asn Leu
 180 185 190

Arg Ser Pro Ala Glu Glu Leu Lys Ala Phe Arg Val Leu Gly Val Ile
 195 200 205

Asp Lys Val Leu Leu Val Met Asp Thr Arg Thr Glu His Thr Phe Ile
 210 215 220

Leu Xaa Gly Leu Arg Lys Ser Ser Glu Tyr Ser Arg Asn Arg Lys Thr
 225 230 235 240

Ile Xaa Pro Arg Cys Val Pro Xaa Met Val Cys Leu His Lys Tyr Ile
 245 250 255

Ile Ser Glu Glu Ser Xaa Phe Leu Val Leu Gln His Ala Glu Xaa Gly
 260 265 270

Lys Leu Trp Ser Tyr Ile Ser Lys Phe Leu Asn Arg Ser Pro Glu Glu
 275 280 285

Ser Phe Asp Ile Lys Glu Val Lys Lys Pro Thr Leu Ala Lys Val His
 290 295 300

Leu Gln Gln Pro Thr Ser Ser Pro Gln Asp Ser Ser Ser Phe Glu Ser
 305 310 315 320

Arg Gly Ser Asp Gly Gly Ser Met Leu Lys Ala Leu Pro Leu Lys Ser
 325 330 335

Ser Leu Thr Pro Ser Ser Gln Asp Asp Ser Asn Gln Glu Asp Asp Gly
 340 345 350

Gln Asp Ser Ser Pro Lys Trp Pro Asp Ser Gly Ser Ser Ser Glu Glu
 355 360 365

Glu Cys Thr Thr Ser Tyr Leu Thr Leu Cys Asn Glu Tyr Gly Gln Glu
 370 375 380

Lys Ile Glu Pro Gly Ser Leu Asn Glu Glu Pro Phe Met Lys Thr Glu
 385 390 395 400

Gly Asn Gly Val Asp Thr Lys Ala Ile Lys Ser Phe Pro Ala His Leu
 405 410 415

Ala Ala Asp Ser Asp Ser Pro Ser Thr Gln Leu Arg Ala His Glu Leu

420	425	430
Lys Phe Phe Pro Asn Asp Asp Pro Glu Ala Val Ser Ser Pro Arg Thr		
435	440	445
Ser Asp Ser Leu Ser Arg Ser Lys Asn Ser Pro Met Glu Phe Phe Arg		
450	455	460
Ile Asp Ser Lys Asp Ser Ala Ser Glu Leu Leu Gly Leu Asp Phe Gly		
465	470	475
Glu Lys Leu Tyr Ser Leu Lys Ser Glu Pro Leu Lys Pro Phe Phe Thr		
485	490	495
Leu Pro Asp Gly Asp Ser Ala Ser Arg Ser Phe Asn Thr Ser Glu Ser		
500	505	510
Lys Val Glu Phe Lys Ala Gln Asp Thr Ile Ser Arg Gly Ser Asp Asp		
515	520	525
Ser Val Pro Val Ile Ser Phe Lys Asp Ala Ala Phe Asp Asp Val Ser		
530	535	540
Gly Thr Asp Glu Gly Arg Pro Asp Leu Leu Val Asn Leu Pro Gly Glu		
545	550	555
Leu Glu Ser Thr Arg Glu Ala Ala Ala Met Gly Pro Thr Lys Phe Thr		
565	570	575
Gln Thr Asn Ile Gly Ile Ile Glu Asn Lys Leu Leu Glu Ala Pro Asp		
580	585	590
Val Leu Cys Leu Arg Leu Ser Thr Glu Gln Cys Gln Ala His Glu Glu		
595	600	605
Lys Gly Ile Glu Glu Leu Ser Asp Pro Ser Gly Pro Lys Ser Tyr Ser		
610	615	620
Ile Thr Glu Lys His Tyr Ala Gln Glu Asp Pro Arg Met Leu Phe Val		
625	630	635
Ala Xaa Val Asp His Ser Ser Ser Gly Asp Met Ser Leu Leu Pro Ser		
645	650	655
Ser Asp Pro Lys Phe Gln Gly Leu Gly Val Val Glu Ser Xaa Val Thr		
660	665	670
Ala Asn Asn Thr Glu Glu Ser Leu Phe Arg Ile Cys Ser Pro Leu Ser		
675	680	685
Gly Ala Asn Glu Tyr Ile Ala Ser Thr Asp Thr Leu Lys Thr Glu Glu		
690	695	700
Val Leu Leu Phe Thr Asp Gln Thr Asp Asp Leu Ala Lys Glu Glu Pro		
705	710	720
Thr Ser Leu Phe Xaa Arg Asp Ser Glu Thr Lys Gly Glu Ser Gly Leu		
725	730	735
Val Leu Glu Gly Asp Lys Glu Ile His Gln Ile Phe Glu Gly Pro		
740	745	750

(2) INFORMATION FOR SEQ ID NO:7:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 6 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS:
 - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Ala Arg Gly Ser Thr Gln
1 5

(2) INFORMATION FOR SEQ ID NO:8:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 16 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS:
 - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Ala Arg Gly Ser Ser Gln Val Arg Val Lys Ser Trp Arg Gly Asp Met
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:9:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 271 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

CCGCACGAGC CTCTGTATG CTTCTTGGCA TGATGGCACG AGGAAAGCCA GAAATTGTGG	60
GAAGCAATTT AGACACACTG ATGAGCATAG GGCTGGATGA GAAGTTTCCA CAGGACTACA	120
GGCTGGCCA GCAGGTGTGC CATGCCATTG CCAACATCTC GGACAGGAGA AAGCCTTCTC	180
TGGGCAAACG TCACCCCCCCC TTCCGGCTGC CTCAGGAACA CAGGTTGTTT GAGCGACTGC	240
GGGAGACAGT CACAAAAGGC TTTGTCCACC C	271

(2) INFORMATION FOR SEQ ID NO:10:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 403 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

GGGTGGATAA CCTGAGGTAG GGAGTCGAG ACCAGCCTGA CCAACATGGA GAAACCCAT	60
CTCTACTAAA AATAAAAAAT TAGCCGGCGT ATTGGCGTGC GCCTGTAATC CCAGCTACTC	120
AAGAGGCTGA GGCAGGGAGAA TCGCCTGAAC CCAGAGGCCGG AGGTTGTAGT GAGCCGAAAT	180
CACACCATTG CACTCCAGCT TGGGCAACAA TAGCGAACCT CCATCTCAA TTAAAAAAAAA	240
AATGCCTACA CGCTTCTTTA AAATGCAAGG CTTTCTCTTA AATTAGCCTA ACTGAACTGC	300
GTTGAGCTGC TTCAACTTG GAATATATGT TTGCCAATCT CCTTGTTC TAATGAATAA	360
ATGTTTTAT ATACTTTAA AAAAAAAA AAAAAAACTC GAG	403

(2) INFORMATION FOR SEQ ID NO:11:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 2276 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

GGAGGGTTGG GCGGCTTGGC GTCGGAGGAG AGCCCCACCC GCGGAGGAAC CCAGCCTTGC	60
CAACGGAGCT GGCGGAGCTC ACTCCTCAGG TCAGGCGGGC GGCGTANAAA ACGCAGCGGA	120
GCCAGGTGAA ACCAAGGCAC CGCCGTGGCT GGCCCCCGAC AGTTCCCTCA GCCGGGAGGT	180
TGGAGGAGCT GAAAACGCCG CGGAGCCCTC GGCCGCCCGA GCAGGGGCTG GACCCCAGCC	240
CTTGCAGCCT CCCTTCTCCT GGCACCCAAG TGCAGTCCTG GCTGCAGAAG GGGCCGCGGG	300
CGCACTGAGT TTCCAACCTC CGTTCAGCCT GTCTGTCTCA GGGTGCAGCC TTAATGAGAG	360
GTGATTCTA AGCTGCTGGG AACCTGAGGT TGTCAAAGGG GCGGCAGGAA ATGGACAGCA	420
GTATAAAACC CAGAAGCAGA ACTTGAAGGT TAAACCACTA GCCCATTCA CAGAATGTT	480
CATCCATTG TGGACCAAAA GATGGAGTTG GTTTTATTT TTAAAAAGAT AATGTTAATG	540
ATCTGATACC ACTACAAATA TTTACGTGAG AAGATTCATG GACTTGTCTT TTGGTTGGAC	600
TGTCACTCAT TTCTGAAAGT TTCTCAGCC ACAATTCTA TTTGAAAATT CAAGTATCAA	660

AGGATACCAAG GTTTAGAATG GTATAATGAT GTATTTGTC TGAGGACTGC AAATTTATA	720
GAGACCACAG TTGGATTCCA GTGATATTCT GCAATCAAAG TGATTTGATA AACCTAATT	780
TGAAGCATT TATATTATA AGCGACATCA AAAGATGGGA GAAAAAAATG GCGATGCAA	840
AACTTCTGG ATGGAGCTAG AAGATGATGG AAAAGTGGAC TTCATTTTG AACAAAGTACA	900
AAATGTGCTG CAGTCACTGA AACAAAAGAT CAAAGATGGG TCTGCCACCA ATAAAAGAATA	960
CATCCAAGCA ATGATTCTAG TGAATGAAGC AACTATAATT AACAGTTCAA CATCAATAAA	1020
GGATCCTATG CCTGTGACTC AGAAGGAACA GGAAAACAAA TCCAATGCAT TTCCCTCTAC	1080
ATCATGTGAA AACTCCTTTC CAGAAGACTG TACATTCTA ACAACAGGAA ATAAGGAAAT	1140
TCTCTCTCTT GAAGATAAAAG TTGTAGACTT TAGAGAAAAA GACTCATCTT CGAATTTATC	1200
TTACCAAAGT CATGACTGCT CTGGTGTCTG TCTGATGAAA ATGCCACTGA ACTTGAAGGG	1260
AGAAAACCT CTGCAGCTGC CAATCAAATG TCACTTCCAA AGACGACATG CAAAGACAAA	1320
CTCTCATTCT TCAGCACTCC ACGTGAGTTA TAAAACCCCT TGTGGAAGGA GTCTACGAAA	1380
CGTGGAGGAA GTTTTCGTT ACCTGCTTGA GACAGAGTGT AACTTTTAT TTACAGATAA	1440
CTTTCTTTC AATAACCTATG TTCAGTTGGC TCGGAATTAC CCAAAGCAAA AAGAAGTTGT	1500
TTCTGATGTG GATATTAGCA ATGGAGTGGG ATCAGTGCCC ATTTCTTCT GTAATGAAAT	1560
TGACAGTAGA AAGCTCCCAC AGTTAACGTA CAGAAAGACT GTGTGGCCTC GAGCATATAA	1620
TCTAACCAAC TTTTCCAGCA TGTTTACTGA TTCCCTGTGAC TGCTCTGAGG GCTGCATAGA	1680
CATAACAAAAA TGTGCATGTC TTCAACTGAC AGCAAGGAAT GCCAAAACCTT CCCCCTTGTC	1740
AAGTGACAAA ATAACCACGT GATATAAATA TAAAAGACTA CAGAGACAGA TTCCCTACTGG	1800
CATTTATGAA TGCAGCCTTT TGTGCAAATG TAATCGACAA TTGTGTCAA ACCGAGTTGT	1860
CCAACATGGT CCTCAAGTGA GGTTACAGGT GTTCAAAACT GAGCAGAAGG GATGGGGTGT	1920
ACGCTGTCTA GATGACATTG ACAGAGGGAC ATTTGTTGC ATTTATTCAG GAAGATTACT	1980
AAGCAGAGCT AACACTGAAA AATCTTATGG TATTGATGAA AACGGGAGAG ATGAGAATAC	2040
TATGAAAAAT ATATTTCAA AAAAGAGGAA ATTAGAAGTT GCATGTTCAAG ATTGTGAAGT	2100
TGAAGTTCTC CCATTAGGAT TGGAAACACA TCCTAGAACT GCTAAAACGT AGAAATGTCC	2160
ACCAAAGTTC AGTAATAATC CCAAGGAGCT TACTATGGAA ACGAAATATG ATAATATTT	2220
AAGAATTCAAG TATCATTCAAG TTATTAGAGA TCCTGAATCC AAGACAGCCA TTTTC	2276

(2) INFORMATION FOR SEQ ID NO:12:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 3114 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

CAGGAGTCCG AACCCTTCAG TCATATAGAC CCAGAGGAGT CAGAGGAGAC CAGGCTTTG	60
AATATCTTAG GACTTATCTT CAAAGGCCA GCAGCTTCCA CACAAGAAAA GAATCCCCGG	120
GAGTCTACAG GAAACATGGT CACAGGACAG ACTGTCTGTA AAAATAAACCA CAATATGTCG	180
GATCCTGAGG AATCCAGGGG AAATGATGAA CTAGTGAAGC AGGAGATGCT GGTACAGTAT	240
CTGCAGGATG CCTACAGCTT CTCCCGGAAG ATTACAGAGG CCATTGGCAT CATCAGCAAG	300
ATGATGTATG AAAACACAAAC TACAGTGGTG CAGGAGGTGA TTGAATNCTT TGTGATGGTC	360
TTCCAATTTG GGGTACCCCA GGCCCTGTTT GGGGTGCGCC GTATGCTGCC TCTCATCTGG	420
TCTAAGGAGC CTGGTGTCCG GGAAGCCGTG CTTAATGCCT ACCGCCAACT CTACCTCAAC	480
CCCAAAGGGG ACTCTGCCAG AGCCAAGGCC CAGGCTTGA TTCAGAAATCT CTCTCTGCTG	540
CTAGTGGATG CCTCGGTTGG GACCATTCAAG TGTCTTGAGG AAATTCTCTG TGAGTTGTC	600
CAGAAGGATG AGTTGAAACC AGCAGTGACC CATCTGCTGT GGGAGCGGGC CACCGAGAAG	660
GTCGCCTGCT GTCCCTCTGGA GCGCTGTTCC TCTGTATGC TTCTTGGCAT GATGGCACGA	720
AGAAAGCCAG AAATTGTGGG AAGCAATTAA GACACACTGA TGAGCATAGG GCTGGATGAG	780
AAGTTCCAC AGGACTACAG GCTGGCCAG CAGGTGTGCC ATGCCATTGC CAACATCTCG	840
GACAGGAGAA AGCCTTCTCT GGGCAAACGT CACCCCCCCT TCCGGCTGCC TCAGGAACAC	900
AGGTTGTTG AGCGACTGCG GGAGACAGTC ACAAAAGGCT TTGTCCACCC AGACCCACTC	960
TGGATCCCCT TCAAAGAGGT GGCAGTGACC CTCATTTACC AACTGGCAGA GGGCCCCGAA	1020
GTGATCTGTG CCCAGATATT GCAGGGCTGT GCAAAACAGG CCCTGGAGAA GCTAGAAGAG	1080
AAGAGAACCA GTCAGGAGGA CCCGAAGGAG TCCCCCGCAA TGCTCCCCAC TTTCCTGTTG	1140
ATGAAACCTGC TGTCCCTGGC TGGGGATGTG GCTCTGCAGC AGCTGGTCCA CTTGGAGCAG	1200
GCAGTGAGTG GAGAGCTCTG CGGGGCCGA GTTCTCCGGG AAGAACAGGA GCACAAGACC	1260
AAAGATCCCA AGGAGAAGAA TACGAGCTCT GAGACCACCA TGGAGGAGGA GCTGGGGCTG	1320
GTTGGGCAA CAGCAGATGA CACAGAGGCA GAACTAATCC GTGGCATCTG CGAGATGGAA	1380
CTGTTGGATG GCAAACAGAC ACTGGCTGCC TTTGTTCCAC TCTTGCTAA AGTCTGTAAC	1440
AACCCAGGCC TCTATAGCAA CCCAGACCTC TCTGCAGCTG CTTCACTTGC CCTTGGCAAG	1500
TTCTGCATGA TCAGTGCCAC TTTCTGCGAC TCCCAGCTTC GTCTTCTGTT CACCATGCTG	1560
GAAAAGTCTC CACTTCCCAT TGTCCGGTCT AACCTCATGG TTGCCACTGG GGATCTGGCC	1620
ATCCGCTTTC CCAATCTGGT GGACCCCTGG ACTCCTCATC TGTATGCTCG CCTCCGGGAC	1680

CCTGCTCAGC AAGTGCAGAA AACAGCGGGG CTGGTGATGA CCCACCTGAT CCTCAAGGAC	1740
ATGGTGAAGG TGAAGGGGCA GGTCAAGTGAG ATGGCGGTGC TGCTCATCGA CCCCCGAGCCT	1800
CAGATTGCTG CCCTGGCCAA GAACTTCTTC AATGAGCTCT CCCACAAGGG CAACGCAATC	1860
TATAATCTCC TTCCAGATAT CATCAGCCGC CTGTCAGACC CCGAGCTGGG GGTGGAGGAA	1920
GAGCCTTCC ACACCATCAT GAAACAGCTC CTCTCCTACA TCACCAAGGA CAAGCAGACA	1980
GAGAGCCTGG TGGAAAAGCT GTGTCAGCGG TTCCGCACAT CCCGAACGTGA GCGGCAGCAG	2040
CGAGACCTGG CCTACTGTGT GTCACAGCTG CCCCTCACAG AGCGAGGCCT CGCGTAAGATG	2100
CTTGACAATT TTGACTGTTT TGGAGACAAA CTGTCAGATG AGTCCATCTT CAGTGCTTTT	2160
TTGTCAGTTG TGGCAAGCT GCGACGTGGG GCCAAGCCTG AGGGCAAGGC TATAATAGAT	2220
GAATTTGAGC AGAACGCTCG GGCCTGTCAT ACCAGAGGTT TGGATGGAAT CAAGGAGCTT	2280
GAGATTGGCC AAGCAGGTAG CCAGAGAGCG CCATCAGCCA AGAAACCATC CACTGGTTCT	2340
AGGTACCAGC CTCTGGCTTC TACAGCCTCA GACAATGACT TTGTCACACC AGAGCCCCGC	2400
CGTACTACCC GTCGGCATCC AAACACCCAG CAGCGAGCTT CAAAAAAGAA ACCCAAAGTT	2460
GTCTTCTCAA GTGATGAGTC CAGTGAGGAA GATCTTCAG CAGAGATGAC AGAACGACGAG	2520
ACACCCAAGA AAACAACCTCC CATTCTCAGA GCATCGGCTC GCAGGCCACAG ATCCTAGGAA	2580
GTCTGTTCCCT GTCCCTCCCTG TGCAGGGTAT CCTGTAGGGT GACCTGGAAT TCGAATTCTG	2640
TTTCCCTTGT AAAATATTG TCTGTCCTT TTTTTAAAAA AAAAAAAAGG CCGGGCACTG	2700
TGGCTCACGC CTGTAATCCC AGCACTTGC GATACCAAGG CGGGTGGATA ACCTGAGGTA	2760
GGGAGTTCGA GACCAGCCTG ACCAACATGG AGAAACCCCA TCTCTACTAA AAATAAAAAA	2820
TTAGCCGGGC GTATTGGCGT GCGCCTGTAA TCCCAGCTAC TCAAGAGGCT GAGGCAGGAG	2880
AATCGCCTGA ACCCAGAGGC GGAGGTTGTA GTGAGCCGAA ATCACACCAT TGCACTCCAG	2940
CTTGGGCAAC AATAGCGAAC CTCCATCTCA ATTAAAAAA AAAATGCCTA CACGCTCTT	3000
AAAATGCAAG GCTTTCTCTT AAATTAGCCT AACTGAACTG CGTTGAGCTG CTTCAACTTT	3060
GGAAATATATG TTTGCCAATC TCCTTGTTT CTAATGAATA AATGTTTTA TATA	3114

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1797 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

CGGCACGAGA TCGACTGGTT GCAAGTAAAA CAGATGGAAA AATAGTACAG TATGAATGTG	60
AGGGGGATAC TTGCCAGGAA GAGAAAATAG ATGCCTTACA GTTAGAGTAT TCATATTTAC	120
TAACAAGCCA GCTGGAATCT CAGCGAATCT ACTGGGAAAA CAAGATAGTT CGGATAGAGA	180
AGGACACAGC AGAGGAAATT AACAAACATGA AGACCAAGTT TAAAGAAACA ATTGAGAAGT	240
GTGATAATCT AGAGCACAAA CTAAATGATC TCCTAAAAGA AAAGCAGTCT GTGGAAAGAA	300
AGTGCACCTCA GCTAACACACA AAAGTGGCCA AACTCACCAA CGAGCTAAA GAGGAGCAGG	360
AAATGAACAA GTGTTGCGA GCCAACCAAG TCCTCCTGCA GAACAAGCTA AAAGAGGAGG	420
AGAGGGTGCT GAAGGAGACC TGTGACCAAA AAGATCTGCA GATCACCAG ATCCAGGAGC	480
AGCTGCGTGA CGTCATGTTAC TACCTGGAGA CACAGCAGAA GATCAACCCTATGCTGCCG	540
AGACCCGGCA GGAAATCCAG GAGGGACAGA TCAACATCGC CATGGCCTCG GCCTCGAGCC	600
CTGCCTCTTC GGGGGGCAGT GGGAAAGTTGC CCTCCAGGAA GGGCCGCAGC AAGAGGGCA	660
AGTGACCTTC AGAGCAACAG ACATCCCTGA GACTGTTCTC CCTGACACTG TGAGAGTGTG	720
CTGGGACCTT CAGCTAAATG TGAGGGTGGG CCCTAATAAG TACAAGTGAG GATCAAGCCA	780
CAGTTGTTG GCTCTTCAT TTGCTAGTGT GTGATGTANT GAATGTAAAG GGTGCTGACT	840
GGAGAGCTGA TAGAAAGGCG CTGCGTTCGA AAAGGTCTTA ANAGTCACT AACCTCACAT	900
TCTAATGACC ATTTTGCCCTT CCTGCTTGGT AGAAGCCCCA ACTCTGCTGT GCATTTTCC	960
ATTGTATTTA TGGAGTTGGC GTATTGACA TTCAGTTCTG GGCTAGGTTT AAGATGTTAA	1020
GTTATTTCTT GTAACCTCAA AGGTAAGGTT ATCTAGCACT AAAGCACCAA ACCTCTCTGA	1080
GGGCATAACA GCTGCTTAA AGAGAGGTTT CCATTGGCTA TTAAGGAGTT ATGAAAACTC	1140
CCTAGCAATA GTGTCAATATC ATTATCATCT CCCCTTCCT CTGGGGAGTG GAAGAATTGC	1200
TTGAATGTTA TCTGAAAAGA GGCCTGGTAG TAAACCAGGC CCTGGCTCTT TACCAGCAGT	1260
CATCTCTCT TGCTCTGGGG CCAGCCAGGA AAAACAAACA ACCCGGGGCA CATTGGTAG	1320
ACTCAGTGTAA GGAAAATGG TGGCAGCTCC ACTGTTTATT TTTGGTAGCT TCGTACGTCA	1380
TTATGAACCG CAATTAAGGA GGAGGCTTAA TGGCTGTTCC CAAACTCAA TCTCAGAGTG	1440
GGTATCCTAG CATCTAGCAA NACTGAGTGG GGAGATTCT CATCCGTGTG AAAATGTAGA	1500
GTGAGGGCTC TGACTAGCTN ATTGTGTATT TTGTTGGTT TAGTATTTTC TAAATGTTA	1560
CAAAATATTG GGCTGCATGT TCAGGTTGCA GCTANAGGGA GCTTGGCAN ATTTCAATT	1620
ACGCTTTCAA GATATAACCA AAAGCTGTTT CTAATCCTA AAATTAGAAT TTCAACAGAN	1680
CCCCCTTCTAG AACAGTCATA TAACGCTTGT GTGGGCCAAC AGANGGGCTG TGTACTCTCT	1740
CTGGAACCAT AAATGTCAA TAATTATCAA CCTGCANTAA TTGAGCAACT TAAATAA	1797

(2) INFORMATION FOR SEQ ID NO:14:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 720 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

TAATCACCAT	CTGTTTTGT	GGGATGTGCT	GCAGCATTTC	CCAAAAAACT	TNACGTGTAA	60
TGTTGCAAAA	TGAATGTACT	CAGACATTNT	TAATTTTAC	TTAGGGCAGA	CCAACTCTT	120
GAGTCTCTCT	TGGACTTATA	TATACAGATA	TCTTAAGAGT	GGGAATGTAA	AGCATAACCT	180
AATTNTCTTT	CCTATAGAGA	TTCTATTTA	TTTAAAATNT	ATTTNTACAC	TAGTTAGAAT	240
CCTGCTGTTT	TGGCCAAGTA	CTTGCTTGC	ATGTCGTGACC	TTGCAGAACG	TGGGGTGGAT	300
CATAGCATAAC	TAATGAAGAG	AATTAGAAGT	AGTTTACAAA	GCTCGCTCAC	TCCTCATTTC	360
TCTGTGATCC	CTTCTATCCA	GTGGCCCCAC	CACCACCTGG	AAAAACAGAT	TTTCAGTAC	420
AGGTGGGATA	AATGCTCTGA	AAGGCTGTGC	CCAGAGGAAT	GAGCAAATAG	GCAAGTGT	480
CCAAACTACT	TGGAGGTTA	CAAAAAATAT	GTCCCAGAAA	AAAAAAAAAT	CTTACCAAGA	540
TACGTAAAGA	AAAAAAAATT	TTTTTTAAA	CAGTCAAAGA	GTCATGTTG	AATTCACAA	600
AATCACATCA	GACAGAAGTT	GT	TTCTTCA	GGAGGGAAAT	GAACCACTTA	660
ACTACCTTGA	ACAATGAAAT	TGAATTAAAA	TAGCCAAACT	TTGAAAAAAA	AAAAAAA	720

(2) INFORMATION FOR SEQ ID NO:15:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 1996 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

CAGAAGTGCA	CGGGTGGCGG	CGGCTGGTTG	CGGGCCGGCG	GC	GGGGCTGGC	GGAGATGGAG	60
GTAACTCAGG	ATCTTGTCA	AGATGGGGTG	GCTTCACCAAG	CTACCCCTGG	GACCGGGAAA	120	
TCTAAGCTGG	AAACATTGCC	CAAAGAAGAC	CTCATCAAGT	TTGCCAAGAA	ACAGATGATG	180	
CTAATACAGA	AAGCTAAATC	AAGGTGTACA	GAATTGGAGA	AAGAAATTGA	AGAAACTCAGA	240	
TCAAAACCTG	TTACTGAAGG	AACTGGTGAT	ATTATTAAGG	CATTAAC	TGA AC	300	
GCTCTTCTTC	TGGAAAAAGC	AGAGACTGAG	CAACAGTGTC	TTTCTCTGAA	AAAGGAAAAT	360	

ATAAAAATGA AGCAAGAGGT TGAGGATTCT GTAACAAAGA TGGGAGATGC ACATAAGGAG	420
TTGGAACAAT CACATATAAA CTATGTGAAA GAAATTGAAA ATTTGAAAAA TGAGTTGATG	480
GCAGTACGTT CCAAATACAG TGAAGACAAA GCTAACCTAC AAAAGCAGCT GGAAGAACAA	540
TGAATACGCA ATTAGAACCT TCAGAACAC TTAAATTCA GAACAACTCT GAAGATAATG	600
TTAAAAAACT ACAAGAAGAG ATTGAGAAAA TTAGGCCAGG CTTTGAGGAG CAAATTTAT	660
ATCTGAAAA GCAATTAGAC GCTACCACTG ATGAAAAGAA GGAAACAGTT ACTCAACTCC	720
AAAATATCAT TGAGGCTAAT TCTCAGCATT ACCAAAAAAA TATTAATAGT TTGCAGGAAG	780
AGCTTTACA GTTGAAAGCT ATACACCAAG AAGAGGTGAA AGAGTTGATG TGCCAGATTG	840
AAGCATCAGC TAAGGAACAT GAAGCAGAGA TAAATAAGTT GAACGAGCTA AAAGAGAACT	900
TAGTAAAACA ATGTGAGGCA AGTAAAAGA ACATCCAGAA CAAATATGAA TGTGAGTTAG	960
AAAATTTAAG GAAAGCCACC TCAAATGCAA ACCAAGACAA TCAGATATGT TCTATTCTCT	1020
TGCAAGAAAA TACATTTGTA GAACAAGTAG TAAATGAAAA AGTCACACAC TTAGAAGATA	1080
CCTTAAAAGA ACTTGAATCT CAACACAGTA TCTTAAAAGA TGAGGTAACT TATATGAATA	1140
ATCTTAAGTT AAAACTTGAA ATGGATGCTC AACATATAAA GGATGAGTTT TTTCATGAAC	1200
GGGAAGACTT AGAGTTAAA ATTAATGAAT TATTACTAGC TAAAGAAGAA CAGGGCTGTG	1260
TAATTGAAAA ATTAAAATCT GAGCTAGCAG GTTAAATAA ACAGTTTGC TATACTGTAG	1320
AACAGCATAA CAGAGAAGTA CAGACTTTA AGGAACAAAC TCAAAAAGAA ATATCAGAAC	1380
TAAATGAGAC ATTTTGTC GATTAGAAA AAGAAAAATT AACATTAATG TTTGAAATAC	1440
AGGGCTTAA GGAACAGTGT GAAAACCTAC AGCAAGAAAA GCAAGAAGCA ATTTAAATT	1500
ATGAGAGTTT ACGAGAGATT ATGGAAATT TACAAACAGA ACTGGGGAA TCTGCTGGAA	1560
AAATAAGTCA AGAGTTCGAA TCAATGAAGC AACAGCAAGC ATCTGATGTT CATGAACTGC	1620
AGCAGAAGCT CAGAACTGCT TTTACTGAAA AAGATGCCCT TCTCGAAACT GTGAATGCC	1680
TCCAGGGAGA AAATGAAAAG TTACTATCTC ACAAGAATT GGTACCAGAA CTTGAAAATA	1740
CCATAAAAGAA CCTTCAAGAA AAGAATGGAG TATACTTAAT TAGTCTCAGT CAAAGAGATA	1800
CCATGTTAAA AGAATTAGAA GGAAAGATAA ATTCTCTTAC TGAGGAAAAA GATGATTTA	1860
TAAATAAAAT GAAAAATTCC CATGAAGAAA TGGATAATTT CCATAAGAAA TGTGAAAGGG	1920
AAGAAAGATT GATTCTTGAA CTTGGGAAGA AAGTAGAGCA AACTATCCAG TACAACAGTG	1980
AACTAGAACAA AAAGGT	1996

(2) INFORMATION FOR SEQ ID NO:16:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 3642 base pairs
 (B) TYPE: nucleic acid

(C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

GTCCCTGCTGA AGCTCACTCA GATTCCCTCA TTGATACCTT TCCTGAGTGT AGTACGGAAG	60
GCTTCTCCAG TGACACTGAT CTGGTATCTC TTACTGTTGA TGTGGATTCT CTTGCTGAGT	120
TAGATGATGG AATGGCTTCC AATCAAATT CTCCCATTAG AACTTTGGT CTCATCTTT	180
CTTCGGATTC TTCAGCACTA GGGGCTGTTG CTTCTGACAG TGAACAGAGC AAAACAGAAAG	240
AAGAACGGGA AAGTCGTAGC CTCTTCCTG GCAGTTAAA GCCGAAGCTT GGCAAGAGAG	300
ATTATTTGGA GAAAGCAGGA GAATTAATAA AGCTGGCTTT AAAAAAGGAA GAAGAAGACG	360
ACTATGAAGC TGCTTCTGAT TTTTATAGGA AGGGAGTTGA TTTACTCCTA GAAGGTGTT	420
AAGGAGAGTC AAGCCCTACC CGTCGAGAAG CTGTGAAGAG AAGAACAGCC GAGTACCTCA	480
TGCAGGGCAGA AAGTATCTCT AGTCTTATG GGAAACCTCA GCTTGATGAT GTATCTCAGC	540
CTCCAGGATC ACTAAGTTCA AGGCCCTTT GGAACCTAAG GAGCCCTGCC GAGGAGCTGA	600
AGGCCCTCAG AGTCCTTGGG GTGATTGACA AGGTTTACT TGTAATGGAC ACAAGGACAG	660
AACACACTTT CATTAAANA GGTCTAAGGA AAAGCAGTGA ATACAGCAGG AACAGAAAGA	720
CCATCCNCCC CCGCTGTGTG CCCANCATGG TGTGTCTGCA TAAGTACATC ATCTCTGAAG	780
AGTCANTATT TCTTGTGCTG CAGCATGCGG AANGTGGCAA ACTGTGGTCA TATATCAGTA	840
AATTTCTAAA CAGAAGTCCT GAAGAAAGCT TTGACATCAA GGAAGTGAAA AAACCTACAC	900
TTGCAAAAGT TCACCTGCAG CAGCCAATT CTAGCCTCA GGACAGCAGT AGCTTGAAT	960
CCAGAGGAAG TGATGGTGG AGCATGCTTA AAGCTCTGCC TTTGAAGAGT AGTCTTACTC	1020
CAAGTTCTCA AGATGACAGC AACCAGGAAG ATGATGGCCA AGATAGCTCT CCAAAGTGGC	1080
CAGATTCTGG TTCAAGTTCA GAAGAAGAAT GTACTACTAG TTATTTAACCA TTATGCAATG	1140
AATATGGGCA AGAAAAGATT GAACCAGGGT CTTTGAATGA GGAGCCCTTC ATGAAGACTG	1200
AAGGGAATGG TGTGATACA AAAGCTATTA AAAGCTTCCC AGCACACCTT GCTGCTGACA	1260
GTGACAGCCC CAGCACACAG CTGAGAGCTC ACGAGCTGAA GTTCTTCCCC AACGATGACC	1320
CAGAAGCAGT TAGTTCTCCA AGAACATCAG ATTCCCTCAG TAGATCAAAA AATAGCCCCA	1380
TGGAATTCTT TAGGATAGAC AGTAAGGATA GCGCAAGTGA ACTCCTGGGA CTTGACTTTG	1440
GAGAAAAATT GTATAGTCTA AAATCAGAAC CTTGAAACC ATTCTTACT CTTCCAGATG	1500
GAGACAGTGC TTCTAGGAGT TTTAATACTA GTGAAAGCAA GGTAGAGTTT AAAGCTCAGG	1560
ACACCATTAG CAGGGGCTCA GATGACTCAG TGCCAGTTAT TTCATTAAA GATGCTGCTT	1620

TTGATGATGT CAGTGGTACT GATGAAGGAA GACCTGATCT TCTTGTAAAT TTACCTGGTG	1680
AATTGGAGTC ACAAAGAGAA GCTGCAGCAA TGGGACCTAC TAAGTTACA CAAACTAATA	1740
TAGGGATAAT AGAAAATAAA CTCTTGAAG CCCCTGATGT TTTATGCCTC AGGCTTAGTA	1800
CTGAACAATG CCAAGCACAT GAGGAGAAAG GCATAGAGGA ACTGAGTGAT CCCTCTGGC	1860
CCAAATCCTA TAGTATAACA GAGAAACACT ATGCACAGGA GGATCCCAGG ATGTTATTTG	1920
TAGCANCTGT TGATCATAGT AGTCAGGAG ATATGTCTT GTTACCCAGC TCAGATCCTA	1980
AGTTCAAGG ACTTGGAGTG GTTGAGTCAN CAGTAACTGC AAACAACACA GAAGAAAGCT	2040
TATTCCGTAT TTGTTAGTCCA CTCTCAGGTG CTAATGAATA TATTGCAAGC ACAGACACTT	2100
TAAAAACAGA AGAAGTATTG CTGTTACAG ATCAGACTGA TGATTTGGCT AAAGAGGAAC	2160
CAACTCTTT ATTCCANAGA GACTCTGAGA CTAAGGGTGA AAGTGGTTA GTGCTAGAAG	2220
GAGACAAGGA AATACATCAG ATTTTGAAG GACCTTGATA AAAAATTAGC ACTANCCTCC	2280
AGGTTTACA TCCCAGAGGG CTGCATTCAA AGNTGGCAG CTGAAATGGT GGTAGCCCTT	2340
NGATGTTTA ACATAGAGAG GGAATTGTGT GCCCGCGATTG AACCCAAACA ANATNTTATT	2400
GAATGATAGA GGACACATTG AGNTAACGTA TTTAGCAGG TGGAGTGAGG TTGAAGATTG	2460
CTGTGACAGC GATGCCATAG AGAGAATGTA CTGTGCCCA GAGGTTGGAG CAATCACTGA	2520
AGAAAATGAA GCCTGTGATT GGTGGAGTTT GGGTGCTGTC CTCTTGAAC TTNTCACTGG	2580
CAAGACTCTG GTTGAATGCC ATCCAGCAGG AATAAAACT CACACTACTT TGAACATGCC	2640
AGAATGTGTC TCTGAAGAGG CTCGCTCACT CATTCAACAG CTCTTGCAGT TCAATCCTCT	2700
GGAACGACTT GGTGCTGGAG TTGCTGGTGT TGAAGATATC AAATCTCATC CATTTTTAC	2760
CCCTGTGGAT TGGCAGAAC TGATGAGATG AACGTAATGC AGGGTTATCT TCACACATTG	2820
TGATCTTCTC TGTGACAGGC ATCTCCAGCA CTGAGGCACC TCTGACTCAC AGTTACTTAT	2880
GGAGCACCAA AGCATTGGA TAAGGACCGT TATAGGAAAT GGGGGGGAAA TGGCTAAAAG	2940
AGAACAAATT GTTTACAATT ACAAGATATT AGCTAATTGT GCCAGGGCT GTTATATACA	3000
TATATACACA ACCAAGGTGT GATCTGAATT TAATCCACAT TTGGTGTGTC AGATGAGTTG	3060
TAAAGCCAAC TGAAAGAGTT CCTTCAAGAA GTTCCCTGTA TAGGAAGCTA GAAGTGTAGA	3120
ATGAAGTTT ACTTGACAGA AGGACCTTA CATGGCAGCT AACAGTGCTT TTTGCTGACC	3180
AGGATTGGTT TATATGATTA AATTAATATT TGCTTAATAA TACACTAAAA GTATATGAAC	3240
AATGTCATCA ATGAAACTTA AAAGCGAGAA AAAAGAATAT ACACATAATT TCTGACGGAA	3300
AACCTGTACC CTGATGCTGT ATAATGTATG TTGAATGTGG TCCCAGATTA TTTCTGTAAG	3360
AAGACACTCC ATGTTGTCAG CTTTGTACTC TTTGTTGATA CTGCTTATTT AGAGAAGGGT	3420
TCATATAAAC ACTCACTCTG TGTCTTCAAC AGCATCTTC TTTCCCCATC TTTCTATTTT	3480

CTGCACCCCTC TGCTTGTCC CTCATATTCT GTTCTTCGA CTCCCTGCTAA CACACATGCA	3540
ACAAAAAAAGG GAAGGGAGTG CTTATTTCCC TTTGTGTAAG GACTAAGAAA TCATGATATC	3600
AAATAAACAT GGTGAAACAT TNANAAAAAA AAAAAAAAAA AA	3642

(2) INFORMATION FOR SEQ ID NO:17:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 1397 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

GTTCAACTCA ATAGAAGATG ACGTTGCCA GCTAGTGTAT GTGGAAAGAG CTGAAGTGCT	60
CAAATCTGAA GATGGCGCCA GCCTCCCAGT GATGGACCTG ACTGAACTCC CCAAGTGCAC	120
GGTGTGTCTG GAGCGCATGG ACGAGTCTGT GAATGGCATC CTCACAACGT TATGTAACCA	180
CATCTTCCAC AGCCAGTGTAC TACAGCGCTG GGACGATACC ACGTGTCTG TTTGCCGGTA	240
CTGTCAAAAG CCCGAGCCAG TAGAAGAAAA TAAGTGTGTT GAGTGTGGTG TTCAGGAAAA	300
TCTTTGGATT TGTTTAATAT GCGGCCACAT AGGATGTGGA CGGTATGTCA GTCGACATGC	360
TTATAAGCAC TTTGAGGAAA CGCAGCACAC GTATGCCATG CAGCTTACCA ACCATCGAGT	420
CTGGGACTAT GCTGGAGATA ACTATGTTCA TCGACTGGTT GCAAGTAAAA CAGATGGAAA	480
AATAGTACAG TATGAATGTG AGGGGGATAC TTGCCAGGAA GAGAAAATAG ATGCCATTACA	540
GTTAGAGTAT TCATATTAC TAACAAGCCA GCTGGAATCT CAGCGAATCT ACTGGGAAAA	600
CAAGATAGTT CGGATAGAGA AGGACACAGC AGAGGAAATT AACAAACATGA AGACCAAGTT	660
TAAAGAAAACA ATTGAGAAGT GTGATAATCT AGAGCACAAA CTAATGATC TCCTAAAAGA	720
AAAGCAGTCT GTGGAAAGAA AGTGCACCTCA GCTAAACACA AAAGTGGCCA AACTCACCAA	780
CGAGCTAAA GAGGAGCAGG AAATGAACAA GTGTTGCCA GCCAACCAAG TCCTCCTGCA	840
GAACAAAGCTA AAAGAGGAGG AGAGGGTGCT GAAGGAGACC TGTGACCAAA AAGATCTGCA	900
GATCACCAG ATCCAGGAGC AGCTGCGTGA CGTCATGTTAC TACCTGGAGA CACAGCAGAA	960
AGATCAACCA TCTGCCTGCC GAGACCCGGC AGGAAATCCA GGAGGGACAG ATCAACATCG	1020
CCATGGCCTC GGCCTCGAGC CCTGCCTCTT CGGGGGGCAG TGGGAAGTTG CCCTCCAGGA	1080
AGGGCCGCAG CAAGAGGGGC AAGTGACCTT CAGAGCAACA GACATCCCTG AGACTGTTCT	1140
CCCTGACACT GTGAGAGTGT GCTGGGACCT TCAGCTAAAT GTGAGGGTGG GCCCTAATAA	1200
GTACAAGTGA GCATCAAGCC ACAGTTGTTT GGCTCTTCA TTTGCTAGTG TGTGATGTAG	1260

TGAATGTAAA GGGTGCTGAC TGGAGAGCTG ATAGAAAGGC GCTGCGTTCG AAAAGGTCTT	1320
AAGAGTCAC TAACCTCACCA TTCTAATGAC CANTTTGCCT TCCTGCTTGG TAGAAGCCCC	1380
ACACTCTGCT GTGCATT	1397

(2) INFORMATION FOR SEQ ID NO:18:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 800 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

CGGTAATTGA GCANACTTAA AATAAGACCT GTGTTGGAAT TTAGTTCCCT CTGAAGAGGT	60
AGAGGGATAG GTTAGTAAGA TGTATTGTTA ACAACAGGT TTTAGTTTT GCTTTATAA	120
TTAGCCACAG GTTTCAAAT GATCACATT CAGAATAGGT TTTAGCCTG TAATTAGGCC	180
TCATCCCCTT TGACCTAAAT GTCTTACATG TTACTTGTCA GCACATCAAC TGTATCACTA	240
ATCACCACATCT GNTTTGTGG GATGTGCTGC AGCATTCCC AAAAAACTTT ACGTGTAATG	300
TTGCAAAATG AATGTACTCA GACATTCTTA ATTTTACTT AGGGCAGACC AACTCTTGA	360
GTCTCTCTTG GACTTATATA TACAGATATC TTAAGAGTGG GAATGAAAG CATAACCTAA	420
TTCTCTTCC TATAGAGATT CTATTTATT TAAAATCTAT TTTACACTA GTTAGAATCC	480
TGCTGTTTG GCCAAGTACT TGTCTGCAT GTCTGACCTT GCAGAAGCTG GGGTGGATCA	540
TAGCATACTA ATGAAGAGAA TTAGAAGTAG TTTACAAAGC TCGCTCACTC CTCATTTCTC	600
TGTGATCCCT TCTATCCAGT GGCCCCACCA CCACCTGGGA AAACAGATTT TTCAGTACAG	660
GTGGGATAAA TGCTCTGAAA GGCTGTGCC AGAGGAATGA GCAAATAGGC AAGTGTTCC	720
AAACTACTTG GAGGTTTACA AAAAATATGT CCCAGAAAAA AAAAAAATCT TACCAAGATA	780
CGTAAAAAAA AAAAAAAAAA	800

(2) INFORMATION FOR SEQ ID NO:19:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 1810 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

GCAGCTCCC	GGTGCCTGTT	AAAAGCTGGA	GGGGGGATAT	GTGATCCAG	GACCAAAAGC	60
CGGGGGCCAG	ACTCATCGGT	TCATTCAACA	ACCACTATT	AGTGCCTGCT	GTGTTCTGCA	120
GGCCCTGCCA	TAGGCCTTG	ATACAGCGGT	GCATAGCGTA	TGAAAAAGAT	CTGTCCTGGC	180
TGAGGCATCCG	TAATATAAAA	ATCTGAAATC	TGAAATGCTC	CAAAATCCTA	AACTTTTGA	240
GTGCTGACAT	TATGCCACAA	ATGGAAAATT	TCATACCTGA	CCTTATGTGG	GTTGCANTCA	300
AAACACAGGT	GCACAAACACC	CAGTTCATGC	AACATCCCCA	ATGGGAAAAA	AGACCCCCCC	360
AGCTCTCTTC	TGCTGCAGTT	TTTCTGCTCA	CACCTGGATT	TCCCCATGCA	TTCCCACAAA	420
AAGTAATTAA	ATGGCATGCG	TGCAGGCTGG	ACACGCCAAC	AACAGGTTTC	CCACAATGCC	480
CCACATGGGG	CCAAGACCTG	TGTGCATTAC	TCATTGCATT	TTTTGCTTA	TTCTCTGCTG	540
TGTGGTATAA	ATATATTGTT	GAAAATGTCA	AAAAGACCTA	AAGATAACCC	TGTGAATATC	600
AGTGATAAGA	AAAAGAGGAA	GCATTTATGT	TTATCTATAG	CACAGAAAGT	CAAGTTGTTG	660
GAGAAACTGG	ACAGTGGTGT	AAGTGTGAAA	CATCTTACAG	AAGAGTATGG	TGTTGGAATG	720
ACCACCATAT	ATGACCTGAA	GAAACAGAAG	GATAAACTGT	TGAAGTTTA	TGCTGAAAGT	780
GATGAGCAGA	TATTAATGAA	AAATAGAAAA	ACACTTCATA	AAGCTAAAAA	TGAAGATCTT	840
GATCGTGTAT	TGAAAGAGTG	GATCCGTCAG	CGTCGCAGTG	AACACATGCC	ACTTAATGGT	900
ATGCTGATCA	TGAAACAAAGC	AAAGATATAT	CACAATGAAC	TAAAAATTGA	GGGAAACTGT	960
GAATATTCAA	CAGGCTGGTT	GCAGAAATT	AAGAAAAGAC	ATGGCATTAA	ATTTTTAAAG	1020
ACTTGTGGCA	ATAAACGCATC	TGCTGGTCAT	GAAGCAACAG	AGAAGTTAC	TGGCAATTTC	1080
AGTAATGATG	ATGAACAAGA	TGGTAACTT	GAAGGATTCA	NTATGTCAAG	TGAGAAAAAA	1140
ATAATGTCTG	ACCTCCTTAC	ATATACAAAA	AATATACATC	CAGAGACTGT	CAGTAAGCTG	1200
GAAGAAGAGG	ATATCTTNA	TGTTTTAAC	AGTAATAATG	AGGCTCCAGT	TGTTCATTC	1260
TTGTCCAATG	GTGAAGTAAC	AAAAATGGTT	CTGAATCAAG	ATGATCATGA	TGATAATGAT	1320
AATGAAGATG	ATGTTAACAC	TGCAGAAAAA	GTGCCTATAG	ACGACATGGT	AAAAATGTGT	1380
GATGGGCTTA	TTAAAGGACT	AGAGCAGCAT	GCATTCTAA	CAGAGCAAGA	AATCATGTCA	1440
GTTTATAAAA	TCAAAGAGAG	ACTTCTAAGA	CAAAAGCAT	CATTAATGAG	GCAGATGACT	1500
CTGAAAGAAA	CATTTAAAAA	AGCCATCCAG	AGGAATGCTT	CTTCCTCTCT	ACAGGACCCA	1560
CTTCTTGGTC	CCTCAACTGC	TTCTGATGCT	TCTTCTCACC	TAAAAATAAA	ATAAAATACA	1620
GTCTACAGTA	ACCTTTAGT	CAAAACAGCA	TCATACTGG	AAACTGAAAG	CCTACTGTTA	1680
TTTGTATTG	TTGCTTAACA	GCTGATACAG	GTATTCTGGT	GACACTACTG	TGCTGGCTTA	1740
CTTAACCTGA	ATACACTATT	TTTTCGTTG	TAAAAAAAAA	AAAAAAANAA	NAAAAAAAA	1800
AAAAAAANANA						1810

(2) INFORMATION FOR SEQ ID NO:20:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 70 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS:
 - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Ala Arg Glu Gly Gly Lys Met Val Leu Glu Ser Thr Met Val Cys Val
1 5 10 15

Asp Asn Ser Glu Tyr Met Arg Asn Gly Asp Phe Leu Pro Thr Arg Leu
20 25 30

Gln Ala Gln Gln Asp Ala Val Asn Ile Xaa Cys His Ser Lys Thr Arg
35 40 45

Ser Asn Pro Glu Asn Asn Val Gly Leu Ile Thr Leu Ala Asn Asp Cys
50 55 60

Glu Val Leu Thr Thr Leu
65 70

(2) INFORMATION FOR SEQ ID NO:21:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 100 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS:
 - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

Ala Arg Glu Ser Thr Met Val Cys Val Asp Asn Ser Glu Tyr Met Arg
1 5 10 15

Asn Gly Asp Phe Leu Pro Thr Arg Leu Gln Ala Gln Gln Asp Ala Val
20 25 30

Asn Ile Val Cys His Ser Lys Thr Arg Ser Asn Pro Glu Asn Asn Val
35 40 45

Gly Leu Ile Thr Leu Ala Asn Asp Cys Glu Val Leu Thr Thr Leu Thr
50 55 60

Pro Asp Thr Gly Arg Ile Leu Ser Lys Leu His Thr Val Gln Pro Lys
65 70 75 80

Gly Lys Ile Thr Phe Cys Thr Gly Ile Arg Val Ala His Leu Ala Leu
85 90 95

Lys His Arg Gln
100

(2) INFORMATION FOR SEQ ID NO:22:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 214 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

CGGCACGAGA AGGTGGCAAG ATGGTGTGG AAAGCACTAT GGTGTGTGTG GACAACAGTG	60
AGTATATGCG CAATGGAGAC TTCTTACCCA CCAGGCTGCA GGCC CAGCAG GATGCTGTCA	120
ACATANTTTG TCATTCAAAG ACCCGCAGCA ACCCTGAGAA CAACGTGGC CTTATCACAC	180
TGGCTAATGA CTGTGAAGTG CTGACCACAC TCAC	214

(2) INFORMATION FOR SEQ ID NO:23:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 375 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

TATGGACACA TTTGAGCCAG CCAAGGAGGA GGATGATTAC GACGTGATGC AGGACCCCGA	60
GTTCCCTTCAG AGTGTCTTAG AGAACCTCCC AGGTGTGGAT CCCAACAAATG AAGCCATTG	120
AAATGNTATG GGCTCCCTGG CCTCCCAGGC CACCAAGGAC GGCAAGAAGG ACAAGAAGGA	180
GGAAGACAAG AAGTGAGACT GGAGGGAAAG GGTAGCTGAG TCTGCTTAGG GGACTGCATG	240
GGAAGCACGG AATATAGGGT TAGATGTGTG TTATCTGTAA CCATTACAGC CTAATAAAAG	300
CTTGGCAACT TTTTAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA	360
AAAAAAAAAC TCGAG	375

(2) INFORMATION FOR SEQ ID NO:24:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 304 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

CGGCACGAGA AAGCACTATG GTGTGTGTGG ACAACAGTGA GTATATGCGG AATGGAGACT	60
TCTTACCCAC CAGGCTGCAG GCCCAGCAGG ATGCTGTCAA CATAGTTGT CATTCAAAGA	120
CCCGCAGCAA CCCTGAGAAC AACGTGGGCC TTATCACACT GGCTAATGAC TGTGAAGTGC	180
TGACCACACT CACCCCAGAC ACTGGCCGTA TCCTGTCCAA GCTACATACT GTCCAACCCA	240
AGGGCAAGAT CACCTTCTGC ACGGGCATCC GCGTTGCCCA TCTGGCTCTG AAGCACCGAC	300
AAGG	304

(2) INFORMATION FOR SEQ ID NO:25:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 22 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS:
 - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

Val Arg Gly Gly Gly Gly Gly Pro Gly Gly Gly Val Gly Gly	
1 5 10 15	
Arg Cys Gly Gly Gly Gly	
20	

(2) INFORMATION FOR SEQ ID NO:26:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 78 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS:
 - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

Ala Arg Ala Ala Arg Ala Lys Ala Gln Ala Leu Ile Gln Asn Leu Ser	
1 5 10 15	
Leu Leu Leu Val Asp Ala Ser Val Gly Thr Ile Gln Cys Leu Glu Glu	
20 25 30	
Ile Leu Cys Glu Phe Val Gln Lys Asp Glu Leu Lys Pro Ala Val Thr	
35 40 45	
Xaa Leu Leu Trp Glu Arg Ala Thr Glu Lys Val Ala Cys Cys Pro Leu	
50 55 60	

Glu Arg Cys Ser Ser Val Met Leu Leu Gly Met Met Ala Arg
 65 70 75

(2) INFORMATION FOR SEQ ID NO:27:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 384 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS:
 - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

Lys Met Val Leu Glu Ser Thr Met Val Cys Val Asp Asn Ser Glu Tyr
 1 5 10 15

Met Arg Asn Gly Asp Phe Leu Pro Thr Arg Leu Gln Ala Gln Gln Asp
 20 25 30

Ala Val Asn Ile Val Cys His Ser Lys Thr Arg Ser Asn Pro Glu Asn
 35 40 45

Asn Val Gly Leu Ile Thr Leu Ala Asn Asp Cys Glu Val Leu Thr Thr
 50 55 60

Leu Thr Pro Asp Thr Gly Arg Ile Leu Ser Lys Leu His Thr Val Gln
 65 70 75 80

Pro Lys Gly Lys Ile Thr Phe Cys Thr Gly Ile Arg Val Ala His Leu
 85 90 95

Ala Leu Lys His Arg Gln Gly Lys Asn His Lys Met Arg Ile Ile Ala
 100 105 110

Phe Val Gly Ser Pro Val Glu Asp Asn Glu Lys Asp Leu Val Lys Leu
 115 120 125

Ala Lys Arg Leu Lys Lys Glu Lys Val Asn Val Asp Ile Ile Asn Phe
 130 135 140

Gly Glu Glu Glu Val Asn Thr Glu Lys Leu Thr Ala Phe Val Asn Thr
 145 150 155 160

Leu Asn Gly Lys Asp Gly Thr Gly Ser His Leu Val Thr Val Pro Pro
 165 170 175

Gly Pro Ser Leu Ala Asp Ala Leu Ile Ser Ser Pro Ile Leu Ala Gly
 180 185 190

Glu Gly Gly Ala Met Leu Gly Leu Gly Ala Ser Asp Phe Glu Phe Gly
 195 200 205

Val Asp Pro Ser Ala Asp Pro Glu Leu Ala Leu Leu Arg Val Ser
 210 215 220

Met Glu Glu Gln Arg Gln Arg Gln Glu Glu Ala Arg Arg Ala Ala
 225 230 235 240

Ala Ala Ser Ala Ala Glu Ala Gly Ile Ala Thr Thr Gly Thr Glu Asp
 245 250 255
 Ser Asp Asp Ala Leu Leu Lys Met Thr Ile Ser Gln Gln Glu Phe Gly
 260 265 270
 Arg Thr Gly Leu Pro Asp Leu Ser Ser Met Thr Glu Glu Gln Ile
 275 280 285
 Ala Tyr Ala Met Gln Met Ser Leu Gln Gly Ala Glu Phe Gly Gln Ala
 290 295 300
 Glu Ser Ala Asp Ile Asp Ala Ser Ser Ala Met Asp Thr Ser Glu Pro
 305 310 315 320
 Ala Lys Glu Glu Asp Asp Tyr Asp Val Met Gln Asp Pro Glu Phe Leu
 325 330 335
 Gln Ser Val Leu Glu Asn Leu Pro Gly Val Asp Pro Asn Asn Glu Ala
 340 345 350
 Ile Arg Asn Ala Met Gly Ser Leu Pro Pro Arg Pro Pro Arg Thr Ala
 355 360 365
 Arg Arg Thr Arg Arg Arg Lys Thr Arg Ser Glu Thr Gly Gly Lys Gly
 370 375 380

(2) INFORMATION FOR SEQ ID NO:28:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 68 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS:
 - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

Ala Arg Asp Ala Tyr Ser Phe Ser Arg Lys Ile Thr Glu Ala Ile Gly
 1 5 10 15
 Ile Ile Ser Lys Met Met Tyr Glu Asn Thr Thr Val Val Gln Glu
 20 25 30
 Val Ile Glu Phe Phe Val Met Val Phe Gln Phe Gly Val Pro Gln Ala
 35 40 45
 Leu Phe Gly Val Arg Arg Met Leu Pro Leu Ile Trp Ser Lys Glu Pro
 50 55 60
 Gly Val Arg Glu
 65

(2) INFORMATION FOR SEQ ID NO:29:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 97 amino acids
 - (B) TYPE: amino acid

- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

Ala Arg Ala Gln Ala Leu Phe Gly Val Arg Arg Met Leu Pro Leu Ile
 1 5 10 15
 Trp Ser Lys Glu Pro Gly Val Arg Glu Ala Val Leu Asn Ala Tyr Arg
 20 25 30
 Gln Leu Tyr Leu Asn Pro Lys Gly Asp Ser Ala Arg Ala Lys Ala Gln
 35 40 45
 Ala Leu Ile Gln Asn Leu Ser Leu Leu Leu Val Asp Ala Ser Val Gly
 50 55 60
 Thr Ile Gln Cys Leu Glu Glu Ile Leu Cys Glu Phe Val Gln Lys Asp
 65 70 75 80
 Glu Leu Lys Pro Ala Val Thr Gln Leu Leu Trp Glu Pro Ala Thr Glu
 85 90 95
 Lys

(2) INFORMATION FOR SEQ ID NO:30:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 116 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS:
 - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

Ala Arg Ala Thr Thr Ala Phe Gly Cys Arg Ile Trp Asn Pro Cys Ala
 1 5 10 15
 Ala Leu Thr Met Lys Gln Ser Ser Asn Val Pro Ala Phe Leu Ser Lys
 20 25 30
 Leu Trp Thr Leu Val Glu Glu Thr His Thr Asn Glu Phe Ile Thr Trp
 35 40 45
 Ser Gln Asn Gly Gln Ser Phe Leu Val Leu Asp Glu Gln Arg Phe Ala
 50 55 60
 Lys Glu Ile Leu Pro Lys Tyr Phe Lys His Asn Asn Met Ala Ser Phe
 65 70 75 80
 Val Arg Gln Leu Asn Met Tyr Gly Phe Arg Lys Val Ile His Ile Asp
 85 90 95

Ser Gly Ile Val Lys Gln Glu Arg Asp Gly Pro Val Glu Phe Gln His
 100 105 110
 Pro Tyr Phe Gln
 115

(2) INFORMATION FOR SEQ ID NO:31:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 124 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS:
 - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

Ala Arg Gly Ala Thr Cys Glu Arg Cys Lys Gly Gly Phe Ala Pro Ala
 1 5 10 15
 Glu Lys Ile Val Asn Ser Asn Gly Glu Leu Tyr His Glu Gln Cys Phe
 20 25 30
 Val Cys Ala Gln Cys Phe Gln Gln Phe Pro Glu Gly Leu Phe Tyr Glu
 35 40 45
 Phe Glu Gly Arg Lys Tyr Cys Glu His Asp Phe Gln Met Leu Phe Ala
 50 55 60
 Pro Cys Cys His Gln Cys Gly Glu Phe Ile Ile Gly Arg Val Ile Lys
 65 70 75 80
 Ala Met Asn Asn Ser Trp His Pro Glu Cys Phe Arg Cys Asp Leu Cys
 85 90 95
 Gln Glu Val Leu Ala Asp Ile Gly Phe Val Lys Asn Ala Gly Arg His
 100 105 110
 Leu Cys Arg Pro Cys His Asn Arg Glu Lys Ala Arg
 115 120

(2) INFORMATION FOR SEQ ID NO:32:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 768 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

TACGAGGAGG AGGAGGAGGA GGCCCCGGAG GAGGAGGCCGT TGGAGGTCGA TGCGGAGGCG	60
GAGGATGAGG AGGCCGAGGC GCCGGAGGAG CCCGAGGCAG CGGAGCAGGA GGAGGCCGGC	120

CGGAGGGCGGC ATGAGACGAG CGTGGCGGCC GCGGCTGCTC GGGGCCGCGC TGGTTGCCA	180
TTGACAGCGG CGTCTGCAGC TCGCTTCAAG ATGGCCGCTT GGCTCGCATT CATTTCCTGC	240
TGAACGACTT TTAACTTCA TTGTCTTTTC CGCCCGCTTC GATGCCTCG CGCCGGCTGC	300
TCTTCCGGG ATTTTTATC AAGCAGAAAT GCATCGAAC ACGAGAATCA AGATCACTGA	360
GCTAAATCCC CACCTGATGT GTGTGCTTG TGGAGGGTAC TTCATTGATG CCACAACCAT	420
AATAGAATGT CTACATTCT TCTGTAAAAC GTGTATTGTT CGTTACCTGG AGACCAGCAA	480
GTATTGTCT ATTGTGATG TCCAAGTTCA CAAGACCAGA CCACTACTGA ATATAAGGTC	540
AGATAAAACT CTCCAAGATA TTGTATACAA ATTAGTTCCA GGGCTTTCA AAAATGAAAT	600
GAAGAGAAGA AGGGATTTT ATGCAGCTCA TCCTTCTGCT GATGCTGCCA ATGGCTCTAA	660
TGAAGATNGA GGAGAGGTTG CAGATGAAGA TAAGAGAATT ATAACGTGATG ATGAGATAAT	720
AAGCTTATCC ATTGAATTCT TTGACCAGAA CAGATTGGAT CGGAAAGT	768

(2) INFORMATION FOR SEQ ID NO:33:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 642 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

TTTAAATAAA CCAGCAGGTT GCTAAAAGAA GGCATTTAT CTAAAGTTAT TTTAATAGGT	60
GGTATAGCAG TAATTTAAA TTTAAGAGTT GCTTTACAG TTAACAATGG AATATGCCTT	120
CTCTGCTATG TCTGAAAATA GAAGNTATTT ATTATGAGCT TNTACAGGTA TTTTAAATA	180
GAGCAAGCAT GTTGAATTAA AAATATGAAT AACCCCACCC ACAATTTC AGTTTATTTT	240
TTGCTTGGT CGAACTTGGT GTGTGTTCAT CACCCATCAG TTATTGTGA GGGTGTTTAT	300
TCTATATGAA TATTGTTCA TGTTGTATG GGAAAATTGT AGCTAACAT TTCATTGTCC	360
CCAGTCTGCA AAAGAACAC AATTCTATTG CTTGTCTTG CTTATAGTCA TAAATCATT	420
ACTTTACAT ATATTGCTGT TACTTCTGCT TTCTTAAAAA ATATAGTAAA GGATGTTTA	480
TGAAGTCACA AGATACATAT ATTTTATTT TGACCTAAAT TTGTACAGTC CCATTGTAAG	540
TGTTGTTCT AATTATAGAT GTAAAATGAA ATTCATTG TAATTGGAAA AAATCCAATA	600
AAAAGGATAT TCATTTAAAA AAAAAAAAAA AAAAAAAAAA AA	642

(2) INFORMATION FOR SEQ ID NO:34:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 236 base pairs

- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

CGGCACGAGC TGCCAGAGCC AAGGCCAGG CTTTGATTCA GAATCTCTCT CTGCTGCTAG	60
TGGATGCCTC GGTTGGGACC ATTCAGTGTC TTGAGGAAAT TCTCTGTGAG TTTGTGCAGA	120
AGGATGAGTT GAAACCAGCA GTGACCCANC TGCTGTGGGA GCGGGCCACC GAGAAAGTCG	180
CCTGCTGTCC TCTGGAACGC TGTT CCTCTG TCATGCTTCT TGGCATGATG GCACGA	236

(2) INFORMATION FOR SEQ ID NO:35:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 333 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

CCGGCGTAT TGGCGTGCAG CTGTAATCCC AGCTAACTCA AGAGGCTGAG GCAGGAGAAT	60
CGCCTGAACC CAGAGGCGGA GGTTGTAGTG AGCCGAAATC ACACCATTGC ACTCCAGCTT	120
GGGCAACAAT AGCGAACCTC CATCTCAAAT TAAAAAAA AATGCCTACA CGCTCTTAA	180
AATGCAAGGC TTTCTTTAA ATTAGCCTAA CTGAAC TGCG TTGAGCTGCT TCAACTTTGG	240
AATATATGTT TGCCAATCTC CTTGTTTCT AATGAATAA TGTTTTATA TACTTTAGA	300
AAAAAAAAA AAAAAAAA AAAAAAAACTC GAG	333

(2) INFORMATION FOR SEQ ID NO:36:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 1272 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

GCAAGATGGT GTTGGAAAGC ACTATGGTGT GTGTGGACAA CAGTGAGTAT ATGCGGAATG	60
GAGACTTCTT ACCCACCAAGG CTGCAGGCC AGCAGGATGC TGTCAACATA GTTTGTCATT	120

CAAAGACCCG CAGCAACCCT GAGAACAAACG TGGGCCTTAT CACACTGGCT AATGACTGTG	180
AAGTGCTGAC CACACTCACCC CAGACACTG GCCGTACCT GTCCAAGCTA CATACTGTCC	240
AACCCAAGGG CAAGATCACC TTCTGCACGG GCATCCCGT GGCCCCTG GCTCTGAAGC	300
ACCGACAAGG CAAGAACATCAC AAGATGCGCA TCATTGCCCT TGTGGGAAGC CCAGTGGAGG	360
ACAATGAGAA GGATCTGGTG AAACTGGCTA AACGCCTCAA GAAGGAGAAA GTAAATGTTG	420
ACATTATCAA TTTTGGGGAA GAGGAGGTGA ACACAGAAAA GCTGACAGCC TTTGTAAACA	480
CGTTGAATGG CAAAGATGGA ACCGGTTCTC ATCTGGTAC AGTGCCTCCT GGGCCCAGTT	540
TGGCTGATGC TCTCATCAGT TCTCCGATTT TGGCTGGTGA AGGTGGTGCC ATGCTGGTC	600
TTGGTGCCAG TGACTTTGAA TTTGGAGTAG ATCCCAGTGC TGATCCTGAG CTGGCCTTGG	660
CCCTTCGTGT ATCTATGGAA GAGCAGCGGC AGCGGCAGGA GGAGGAGGCC CGGCGGGCAG	720
CTGCAGCTTC TGCTGCTGAG GCCGGGATTG CTACGACTGG GACTGAAGAC TCAGACGATG	780
CCCTGCTGAA GATGACCATC AGCCAGCAAG AGTTTGGCCG CACTGGGCTT CCTGACCTAA	840
GCACTATGAC TGAGGAAGAG CAGATTGCTT ATGCCATGCA GATGTCCTG CAGGGAGCAG	900
AGTTTGGCCA GGCGGAATCA GCAGACATTG ATGCCAGCTC AGCTATGGAC ACATCTGAGC	960
CAGCCAAGGA GGAGGATGAT TAGACAGTGA TGCAGGACCC CGAGTTCCCTT CAGAGTGTCC	1020
TAGAGAACCT CCCAGGTGTG GATCCAACA ATGAAGCCAT TCGAAATGCT ATGGGCTCCC	1080
TGCCCTCCCAG GCCACCAAGG ACGGCAAGAA GGACAAGAAG GAGGAAGACA AGAAGTGAGA	1140
CTGGAGGGAA AGGGTAGCTG AGTCTGCTTA GGGGACTGCA TGGGAAGCAC GGAATATAGG	1200
GTAGATGTG TGTTATCTGT AACCAATTACA GCCTAAATAA AGCTTGGCAA CTTTAAAAAA	1260
AAAAAAAAAA AA	1272

(2) INFORMATION FOR SEQ ID NO:37:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 206 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

CGGCACGAGA TGCCTACAGC TTCTCCCGGA AGATTACAGA GGCCATTGGC ATCATCAGCA	60
AGATGATGTA TGAAAACACA ACTACAGTGG TGCAGGAGGT GATTGAATTC TTTGTGATGG	120
TCTTCCAATT TGGGGTACCC CAGGCCCTGT TTGGGGTGC CGTATGCTG CCTCTCATCT	180
GGTCTAAGGA GCCTGGTGTG CGGGAA	206

(2) INFORMATION FOR SEQ ID NO:38:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 341 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

TACTAAAAAT	AAAAAAATTAG	CCGGCGTAT	TGGCGTGC	CTGTAATCCC	AGCTACTCAA	60	
GAGGCTGAGG	CAGGAGAAC	T	GCCTGAACCC	AGAGGCGGAG	GTTGTAGTGA	GCCGAAATCA	120
CACCATTGCA	CTCCAGCTTG	GGCAACAATA	CGAACCTCC	ATCTCAAATT	AAAAAAAAAA		180
TGCCTACACG	CTCTTTAAA	TGCAAGGCTT	TCTCTTAAAT	TAGCCTAACT	GAACTGCGTT		240
GAGCTGCTTC	AAC	TTGGAA	TATATGTTG	CCAATCTCCT	TGTTTCTAA	TGAATAAATG	300
TTTTTATATA	CTTTTAANGA	GAGAAAAAAA	ANAAACTCGA	G			341

(2) INFORMATION FOR SEQ ID NO:39:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 293 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

CGGCACGAGC	CCAGGCCCTG	TTTGGGGTGC	GCCGTATGCT	GCCTCTCATC	TGGTCTAAGG	60
AGCCTGGTGT	CCGGGAAGCC	GTGCTTAATG	CCTACCGCCA	ACTCTACCTC	AACCCCAAAG	120
GGGACTCTGC	CAGAGCCAAG	GCCCAGGCTT	TGATTCAGAA	TCTCTCTCTG	CTGCTAGTGG	180
ATGCCTCGGT	TGGGACCATT	CAGTGTCTTG	AGGAAATTCT	CTGTGAGTTT	GTGCAGAAGG	240
ATGAGTTGAA	ACCAGCAGTG	ACCCAGCTGC	TGTGGGAACC	GGCCACCGAG	AAA	293

(2) INFORMATION FOR SEQ ID NO:40:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 350 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

CGGCACGAGC TACCACCGCG TTGGGTGTA GAATTTGAA TCCCTGCGCC GCGTTAACAA	60
TGAAGCAGAG TTCGAACGTG CCGGCTTCC TCAGCAAGCT GTGGACGCTT GTGGAGGAAA	120
CCCACACTAA CGAGTTCATC ACCTGGAGCC AGAATGCCA AAGTTTCTG GTCTGGATG	180
AGCAACGATT TGCAAAAGAA ATTCTTCCCA AATATTCAA GCACAATAAT ATGGCAAGCT	240
TTGTGAGGCA ACTGAATATG TATGGTTCC GTAAAGTAAT ACATATCGAC TCTGGAATTG	300
TTAAGCAAGA AAGAGATGGT CCTGTAGAAT TTCAGCATCC TTACTTCAA	350

(2) INFORMATION FOR SEQ ID NO:41:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 377 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

TCCTAAAGCT TTCTCTGCTC CAGTTATTT TATTAATAT TTTCACTTG GCTTATTTT	60
AAAACGGGA ACATAAAAGTG CCTGTATCTT GTAAAACCTTC ATTTGTTCT TTTGGTTCA	120
AGAAGTCAT TTATGTTCAA AGACGTTAT TCATGTTCAA CAGGAAAGAC AAAGTGTACG	180
TGAATGCTCG CTGTCTGATA GGGTCCAGC TCCATATATA TAGAAAGATC GGGGGTGGGA	240
TGGGATGGAG TGAGCCCCAT CCAGTTAGTT GGACTAGTT TAAATAAAGG TTTCCGGTT	300
TGTGTTTTT TGAACCATAAC TGTTTAGTAA AATAAATACA ATGAATGTTG NAAAAAAA	360
AAAAAAAAAA ACTCGAG	377

(2) INFORMATION FOR SEQ ID NO:42:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 374 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

CGGCACGAGG CGCCACTTGC GAGCGCTGCA AGGGCGGCTT TGCGCCCGCT GAGAAGATCG	60
TGAACAGTAA TGGGGAGCTG TACCATGAGC AGTGTTCGT GTGCGCTCAG TGCTTCCAGC	120
AGTTCCCAGA AGGACTCTTC TATGAGTTG AAGGAAGAAA GTACTGTGAA CATGACTTTC	180

AGATGCTCTT TGCCCCTTGC TGTCACTAGT GTGGTGAATT CATCATTGGC CGAGTTATCA	240
AAGCCATGAA TAACAGCTGG CATCCGGAGT GCTTCCGCTG TGACCTCTGC CAGGAAGTTC	300
TGGCAGATAT CGGGTTTGTG AAGAATGCTG GGAGACACCT GTGTGCCCC TGTCATAATC	360
GTGAGAAAGC CAGA	374

(2) INFORMATION FOR SEQ ID NO:43:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 492 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

CTTGCATTT TACAGTAAGA ATCAAAGTCC CTTCAAGTGTG CCTTTGTCAG CTAATATGTG	60
ACCAGCAATG ACAACCTTGG GAGTATTTAT TAAATATTAT GCTATGAATA TAGGCAACAC	120
AGAACAGGGT TTGCAGTATA GCGTCTTGAT GCTAAATTCT CATATACCTC TACACGAGAA	180
ATATGGAGGA GAAAAACAAG CATTACATA TATTCTTCGT CACTTGAAAG ATGCATGACC	240
TGAACACTGAC TGCTTGTGTT TGTTTACATA TCAGGCATAC CCAGGCATCT CCTGCAGCCA	300
GAGGTTCCAT TGCTGTCTT GCTCAGTCCT CTTTAAAT ATGAATTAGT GGACAGGCAC	360
GGTGCCTCAC ACCTGTAATC CCAGCACTTT GGGAGGTCGA GGCAGGTGGA TCACGAGGTC	420
AGGAGATCAA GACCATCTG GCTACCACTG AAACCCCATC TCTACTACAA AAAAAAAAAA	480
AAAAAAACTCG AG	492

(2) INFORMATION FOR SEQ ID NO:44:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 15 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

Ser Gln Ile Cys Glu Leu Val Ala His Glu Thr Ile Ser Phe Leu	
1 5 10 15	

(2) INFORMATION FOR SEQ ID NO:45:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 15 amino acids

- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

Xaa Xaa Xaa Xaa Xaa Ser Ile Leu Asp Glu Val Ile Arg Gly Thr
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:46:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 21 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

Val Val Lys Thr Tyr Leu Ile Ser Ser Ile Pro Gln Gly Ala Phe Asn
1 5 10 15

Tyr Lys Tyr Thr Ala
20

(2) INFORMATION FOR SEQ ID NO:47:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 21 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

Val Val Lys Thr Tyr Leu Ile Ser Ser Ile Pro Leu Gln Ala Phe Asn
1 5 10 15

Tyr Lys Tyr Thr Ala
20

(2) INFORMATION FOR SEQ ID NO:48:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 15 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

Xaa Ala Lys Lys Phe Leu Asp Ala Glu His Lys Leu Asn Phe Ala
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:49:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 15 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

Xaa Xaa Xaa Lys Ile Lys Lys Phe Ile Gln Glu Asn Ile Phe Gly
 1 5 10 15

(2) INFORMATION FOR SEQ ID NO:50:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 18 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:

Val Thr

(2) INFORMATION FOR SEQ ID NO:51:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 15 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

Xaa Tyr Gln Tyr Pro Ala Leu Thr Xaa Glu Gln Lys Lys Glu Leu
 1 5 10 15

(2) INFORMATION FOR SEQ ID NO:52:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 14 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:52:

Xaa Pro Ala Val Tyr Phe Lys Xaa Xaa Phe Leu Asp Xaa Asp
1 5 10

(2) INFORMATION FOR SEQ ID NO:53:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 15 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:53:

Xaa Pro Ala Val Tyr Phe Lys Glu Gln Phe Leu Asp Gly Asp Gly
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:54:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 18 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:54:

Xaa Xaa Val Ala Val Leu Xaa Ala Ser Xaa Xaa Ile Gly Gln Pro Leu
1 5 10 15
Ser Leu

(2) INFORMATION FOR SEQ ID NO:55:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 15 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:55:

Val Val Lys Thr Tyr Leu Ile Ser Xaa Ile Pro Leu Gln Gly Ala
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:56:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 15 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:56:

Xaa Xaa Lys Thr Tyr Leu Ile Ser Ser Ile Pro Leu Gln Gly Ala
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:57:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 15 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:57:

Met Asp Ile Pro Gln Thr Lys Gln Asp Leu Glu Leu Pro Lys Leu
1 5 10 15

CLAIMS

1. A polypeptide comprising an immunogenic portion of a prostate protein having a partial sequence selected from the group consisting of SEQ ID Nos. 2, 4, 5, 6, 7 and 8, or a variant of said protein that differs only in conservative substitutions and/or modifications.
2. A polypeptide comprising an immunogenic portion of a prostate protein or a variant of said protein that differs only in conservative substitutions and/or modifications wherein said protein comprises an amino acid sequence of a portion thereof encoded by a DNA sequence selected from the group consisting of the sequences recited in SEQ ID Nos. 11 and 13-19, the complements of said sequences, and DNA sequences that hybridize to a sequence recited in SEQ ID Nos. 11 and 13-19, or a complement thereof under moderately stringent conditions.
3. A DNA molecule comprising a nucleotide sequence encoding the polypeptide of claims 1 or 2.
4. An expression vector comprising the DNA molecule of claim 3.
5. A host cell transformed with the expression vector of claim 4.
6. The host cell of claim 5 wherein the host cell is selected from the group consisting of *E. coli*, yeast and mammalian cell lines.
7. A pharmaceutical composition comprising the polypeptide of claims 1 or 2 and a physiologically acceptable carrier.
8. A vaccine comprising the polypeptide of claims 1 or 2 and a non-specific immune response enhancer.

9. The vaccine of claim 8 wherein the non-specific immune response enhancer is an adjuvant.

10. A vaccine comprising a DNA molecule and a non-specific immune response enhancer, the DNA molecule comprising a nucleotide sequence encoding the polypeptide of claims 1 or 2.

11. The vaccine of claim 10 wherein the non-specific immune response enhancer is an adjuvant.

12. A pharmaceutical composition for the treatment of prostate cancer comprising a polypeptide and a physiologically acceptable carrier, the polypeptide comprising an immunogenic portion of a prostate protein having a partial sequence selected from the group consisting of SEQ ID Nos. 1, 3, 20, 21, 25-31 and 44-57.

13. A vaccine for the treatment of prostate cancer comprising a polypeptide and a non-specific immune response enhancer, the polypeptide comprising an immunogenic portion of a prostate protein having a partial sequence selected from the group consisting of SEQ ID Nos. 1, 3, 20, 21, 25-31 and 44-57.

14. The vaccine of claim 13 wherein the non-specific immune response enhancer is an adjuvant.

15. A pharmaceutical composition according to claim 7, for use in the manufacture of a medicament for inhibiting the development of prostate cancer.

16. A vaccine according to claim 8, for use in the manufacture of a medicament for inhibiting the development of prostate cancer.

17. A method for detecting prostate cancer in a patient, comprising:
 - (a) contacting a biological sample obtained from a patient with a binding agent which is capable of binding to the polypeptide of claims 1 or 2; and
 - (b) detecting in the sample a protein or polypeptide that binds to the binding agent, thereby detecting prostate cancer in the patient.
18. The method of claim 17 wherein the binding agent is a monoclonal antibody.
19. The method of claim 17 wherein the binding agent is a polyclonal antibody.
20. A method for monitoring the progression of prostate cancer in a patient, comprising:
 - (a) contacting a biological sample obtained from a patient with a binding agent that is capable of binding to the polypeptide of claims 1 or 2;
 - (b) determining in the sample an amount of a protein or polypeptide that binds to the binding agent;
 - (c) repeating steps (a) and (b); and
 - (d) comparing the amount of polypeptide detected in steps (b) and (c) to monitor the progression of prostate cancer in the patient.
21. A method for detecting prostate cancer in a patient, comprising:
 - (a) contacting a biological sample obtained from a patient with a binding agent which is capable of binding to a polypeptide, the polypeptide comprising an immunogenic portion of a prostate protein having a partial sequence selected from the group consisting of SEQ ID Nos. 1, 3, 20, 21, 25-31 and 44-57; and
 - (b) detecting in the sample a protein or polypeptide that binds to the binding agent, thereby detecting prostate cancer in the patient.

22. The method of claim 21 wherein the binding agent is a monoclonal antibody.

23. The method of claim 21 wherein the binding agent is a polyclonal antibody.

24. A method for monitoring the progression of prostate cancer in a patient, comprising:

(a) contacting a biological sample obtained from a patient with a binding agent that is capable of binding to a polypeptide, the polypeptide comprising an immunogenic portion of a prostate protein having a partial sequence selected from the group consisting of: SEQ ID Nos. 1, 3, 20, 21, 25-31 and 44-57;

(b) determining in the sample an amount of a protein or polypeptide that binds to the binding agent;

(c) repeating steps (a) and (b); and

(d) comparing the amount of polypeptide detected in steps (b) and (c) to monitor the progression of prostate cancer in the patient.

25. A monoclonal antibody that binds to the polypeptide of claims 1 or 2.

26. A monoclonal antibody according to claim 25, for use in the manufacture of a medicament for inhibiting the development of prostate cancer.

27. The monoclonal antibody of claim 26 wherein the monoclonal antibody is conjugated to a therapeutic agent.

28. A method for detecting prostate cancer in a patient, comprising:

(a) contacting a biological sample from a patient with at least two oligonucleotide primers in a polymerase chain reaction, wherein at least one of the

oligonucleotide primers is specific for a DNA molecule selected from the group consisting of SEQ ID Nos. 9-19, 22-24 and 32-43; and

(b) detecting in the sample a DNA sequence that amplifies in the presence of the oligonucleotide primer, thereby detecting prostate cancer.

29. The method of claim 28, wherein at least one of the oligonucleotide primers comprises at least about 10 contiguous nucleotides of a DNA molecule selected from the group consisting of SEQ ID Nos. 9-19, 22-24 and 32-43.

30. A method for detecting prostate cancer in a patient, comprising:

(a) contacting a biological sample from the patient with at least one oligonucleotide probe specific for a DNA molecule selected from the group consisting of SEQ ID Nos. 9-19, 22-24 and 32-43; and

(b) detecting in the sample a DNA sequence that hybridizes to the oligonucleotide probe, thereby detecting prostate cancer.

31. The method of claim 30 wherein the probe comprises at least about 15 contiguous nucleotides of a DNA molecule selected from the group consisting of SEQ ID Nos. 9-19, 22-24 and 32-43.

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Rat Prostate Extracts

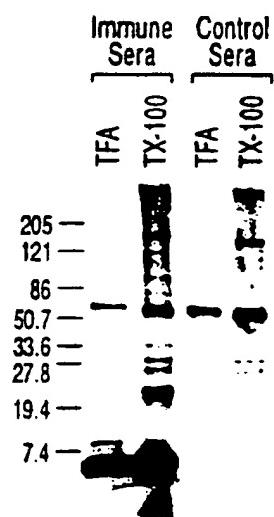


Fig. 1

Rat Prostate Extract

Non-reduced SDS-PAGE

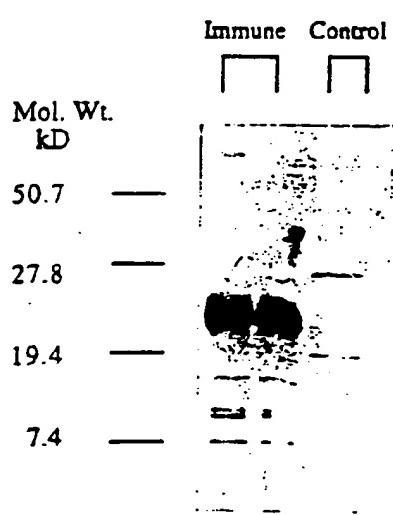


Fig. 2

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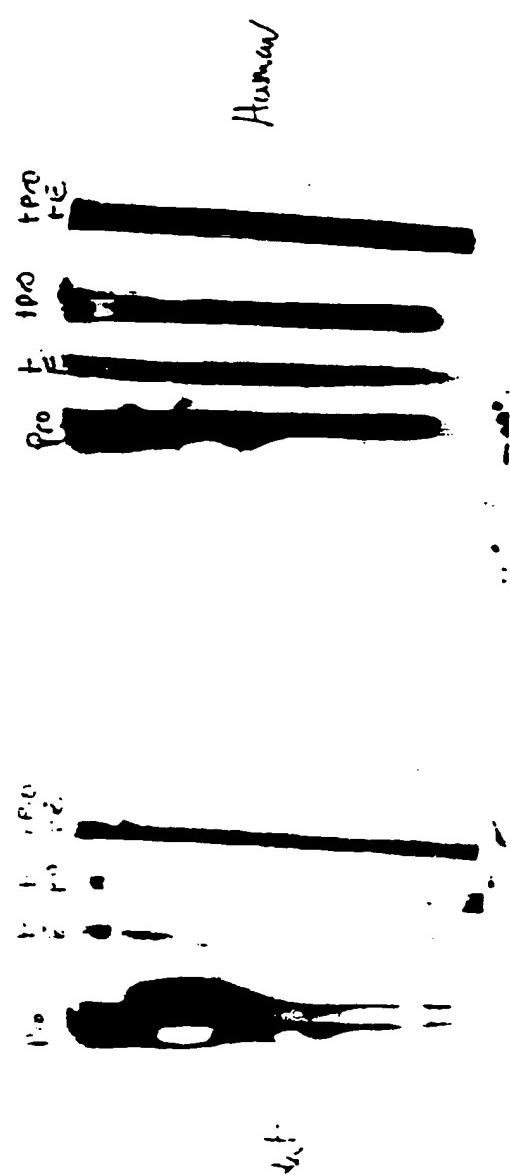


Fig. 3

**CORRECTED
VERSION***

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International Bureau



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C12N 15/12, A61K 38/17, 39/00, C12Q 1/68, G01N 33/68, C07K 16/18, 14/47, A61K 39/395 C12N 1/21	A3	(11) International Publication Number: WO 97/33909 (43) International Publication Date: 18 September 1997 (18.09.97)
(21) International Application Number: PCT/US97/04192		(81) Designated States: AL, AM, AT, AU, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, UZ, VN, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).
(22) International Filing Date: 14 March 1997 (14.03.97)		
(30) Priority Data: 08/616,745 15 March 1996 (15.03.96) US 08/633,840 11 April 1996 (11.04.96) US		
(71) Applicant: CORIXA CORPORATION [US/US]; Suite 464, 1124 Columbia Street, Seattle, WA 98104 (US).		Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
(72) Inventors: REED, Steven, G.; 2843 122nd Place N.E., Bellevue, WA 98005 (US). DILLON, Davin, C.; 21607 N.E. 24th Street, Redmond, WA 98053 (US). TWARDZIK, Daniel, R.; 10195 South Beach Drive, Bainbridge Island, WA 98110 (US).		
(74) Agents: MAKI, David, J. et al.; Seed and Berry L.L.P., 6300 Columbia Center, 701 Fifth Avenue, Seattle, WA 98104-7092 (US).		(88) Date of publication of the international search report: 24 December 1997 (24.12.97)

(54) Title: COMPOUNDS AND METHODS FOR IMMUNOTHERAPY AND IMMUNODIAGNOSIS OF PROSTATE CANCER

(57) Abstract

Compounds and methods for treating and diagnosing prostate cancer are provided. The inventive compounds include polypeptides containing at least a portion of a prostate protein. Vaccines and pharmaceutical compositions for immunotherapy of prostate cancer comprising such polypeptides or DNA molecules encoding such polypeptides are also provided. The inventive polypeptides may also be used to generate antibodies useful for the diagnosis and monitoring of prostate cancer. Nucleic acid sequences for preparing probes, primers, and polypeptides are also provided.

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INTERNATIONAL SEARCH REPORT

Inten. /inal Application No
PCT/US 97/04192

A. CLASSIFICATION OF SUBJECT MATTER					
IPC 6	C12N15/12	A61K38/17	A61K39/00	C12Q1/68	G01N33/68
	C07K16/18	C07K14/47	A61K39/395	C12N1/21	

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C07K C12N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>WO 94 09820 A (SLOANKETTERING INSTITUTE FOR CANCER RESEARCH) 11 May 1994</p> <p>see the whole document especially see page 43 - page 44 see page 54 - page 60 see page 86 see page 51 - page 53 see sequences ID 1 and ID 2</p> <p>---</p>	1-11, 15-20, 25-31
A	<p>EL-SHIRBINY A M: "PROSTATIC SPECIFIC ANTIGEN" ADVANCES IN CLINICAL CHEMISTRY, vol. 31, 1994, pages 99-133, XP000617158 see the whole document</p> <p>---</p> <p>-/-</p>	1-11, 15-20, 25-31

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

* Special categories of cited documents :

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- *L* document which may throw doubt on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

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- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
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- *Z* document member of the same patent family

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Date of the actual completion of the international search

2 July 1997

Date of mailing of the international search report

30.10.97

Name and mailing address of the ISA
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INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 97/04192

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 95 04548 A (JENNER TECHNOLOGIES) 16 February 1995 see the whole document -----	1-11

1

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 97/04192

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:

2. Claims Nos.: because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see continuation-sheet

1. As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-11; 15-20; 25-31 all partially (invention 1.)

Remark on Protest

- The additional search fees were accompanied by the applicant's protest.
 No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/10

1. Claims (1-11) partially; (15-20) partially; 25-31 (partially)
Prostate protein characterized by sequence ID2 and its nucleic acid sequence (ID11), expression vector, host cell, use of the protein in a pharmaceutical composition, antibody against the protein and its use in a method of diagnosis of prostate cancer and in a method of monitoring the progression of prostate cancer. Method of detection of prostate cancer using primers and probes derived from the nucleic acid sequence.
2. Claims (1-11) partially; (15-20) partially; (25-31) partially
The same as defined above but for sequences ID4 and ID13, 14.
3. Claims (1-11) partially; (15-20) partially; (25-31) partially
The same as defined above but for sequences ID5 and ID15
4. Claims (1-11) partially; (15-20) partially; (25-31) partially
The same as defined above but for sequences ID6 and ID16
5. Claims (1-11) partially; (15-20) partially; (25-31) partially
The same as defined above but for sequences ID7 and ID17, 18
6. Claims (1-11) partially; (15-20) partially; (25-31) partially
The same as defined above but for sequences ID8 and ID19
7. Claims (12-14) partially; (21-24) partially
Pharmaceutical composition containing a prostate protein defined by sequence ID1. A method for detecting prostate cancer and a method for monitoring the progression of Prostate Cancer using an antibody against the prostate protein.
8. Claims (12-14) partially; (21-24) partially
The same as defined above but for sequence ID3
9. Claims (12-14) partially; (21-24) partially
The same as defined above but for sequence ID20
10. Claims (12-14) partially; (21-24) partially
The same as defined above but for sequence ID21
11. Claims (12-14) partially; (21-24) partially
The same as defined above but for sequence ID25
12. Claims (12-14) partially; (21-24) partially
The same as defined above but for sequence ID26
13. Claims (12-14) partially; (21-24) partially
The same as defined above but for sequence ID 27
14. Claims (12-14) partially; (21-24) partially
The same as defined above but for sequence ID 28
15. Claims (12-14) partially; (21-24) partially
The same as defined above but for sequence ID 29
16. Claims (12-14) partially; (21-24) partially
The same as defined above but for sequence ID 30

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FURTHER INFORMATION CONTINUED FROM PCT/ISA/10

17. Claims (12-14) partially; (21-24) partially
The same as defined above but for sequence ID31
18. Claims (12-14) partially; (21-24) partially
The same as defined above but for sequences ID44 and ID45
19. Claims (12-14) partially; (21-24) partially
The same as defined above but for sequences ID46 and ID47
20. Claims (12-14) partially; (21-24) partially
The same as defined above but for sequences ID48 and ID49
21. Claims (12-14) partially; (21-24) partially
The same as defined above but for sequence ID50
22. Claims (12-14) partially; (21-24) partially
The same as defined above but for sequence ID51
23. Claims (12-14) partially; (21-24) partially
The same as defined above but for sequence ID52
24. Claims (12-14) partially; (21-24) partially
The same as defined above but for sequence ID53
25. Claims (12-14) partially; (21-24) partially
The same as defined above but for sequence ID54
26. Claims (12-14) partially; (21-24) partially
The same as defined above but for sequences ID55 and ID56
27. Claims (12-14) partially; (21-24) partially
The same as defined above but for sequence ID57

INTERNATIONAL SEARCH REPORT

Information on patent family members

Int'l Application No

PCT/US 97/04192

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9409820 A	11-05-94	CA 2147499 A EP 0668777 A JP 8506005 T US 5538866 A	11-05-94 30-08-95 02-07-96 23-07-96
WO 9504548 A	16-02-95	AU 7631294 A EP 0721345 A JP 9504000 T	28-02-95 17-07-96 22-04-97